Product Development Under the Animal Rule

Guidance for Industry

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)

October 2015
Animal Rule

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Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not create any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance[2] provides information and recommendations on drug and biological product[3] development when human efficacy studies are not ethical or feasible. The regulations that set forth the pathway for approval[4] of these products under 21 CFR 314.600 through 314.650 (drugs) or 21 CFR 601.90 through 601.95 (biological products) are commonly referred to as the *Animal Rule*.

This guidance does not address the following topics:

- The chemistry, manufacturing, and controls or nonclinical pharmacology and toxicology studies necessary for drug development
- Issues related to initial proof-of-concept studies
- The details of study design and conduct for drug-specific animal efficacy studies or for human pharmacokinetic (PK) and/or safety studies
- Drug development in specific populations (e.g., pediatrics, geriatrics, and pregnant women)[5]
- The development of combination products

[1] This guidance has been prepared by 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. The Office of Good Clinical Practice and the Office of Counterterrorism and Emerging Threats also provided input.

[2] This guidance finalizes the 2014 revised draft guidance for industry *Product Development Under the Animal Rule*, which replaced the 2009 draft guidance for industry *Animal Models – Essential Elements to Address Efficacy Under the Animal Rule*.

[3] As used in this guidance, all references to *drugs* include human drugs, therapeutic biological products, cellular and gene therapies, and vaccines, unless otherwise specified.

[4] As used in this guidance, the term *approval* refers to approval or licensure.

[5] The Animal Rule applies equally to adult and pediatric populations.

- Requirements for procurement of medical countermeasures by the federal government (e.g., Strategic National Stockpile (SNS)[6])
- The development of animal models for other purposes, such as for assessment of toxicology

Information on FDA guidances is available on FDA's Web page.[7] In addition, FDA guidances related to medical countermeasures for chemical, biological, radiological, and nuclear (CBRN) agents can be accessed through FDA's Medical Countermeasures Initiative (MCMi) Web page.[8]

In general, FDA's guidance documents 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.

II. THE ANIMAL RULE

FDA's regulations concerning the approval of new drugs when human efficacy studies are not ethical[9] and field trials are not feasible are codified in 21 CFR 314.600 through 314.650 for drugs and 21 CFR 601.90 through 601.95 for biological products. Approval under the Animal Rule can be pursued only if human efficacy studies cannot be conducted because the conduct of such trials is unethical and field trials after an accidental or deliberate exposure are not feasible. The Animal Rule does not apply to drugs that can be approved for the proposed indication "based on efficacy standards described elsewhere in FDA's regulations . . . ."[10]

The Animal Rule states that for drugs developed to ameliorate or prevent serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic substances, when human efficacy studies are not ethical and field trials are not feasible, FDA may grant marketing approval based on adequate and well-controlled animal efficacy studies when the results of those studies establish that the drug is reasonably likely to produce clinical benefit in humans. Drugs evaluated for efficacy under the Animal Rule should be evaluated for safety under the existing requirements for establishing the safety of new drugs. The Animal Rule states

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[6] Sponsors should discuss issues related to the SNS with the Department of Health and Human Services/Biomedical Advanced Research and Development Authority (HHS/BARDA) and the Centers for Disease Control and Prevention (CDC).

[7] FDA guidances are updated periodically. The most recent versions are available at http://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

[8] The MCMi Web page is available at http://www.fda.gov/emergencypreparedness/medicalcountermeasures/default.htm.

[9] As described in the Scope of the Animal Rule, " . . . it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance . . . ." See 21 CFR 314.600 for drugs and 21 CFR 601.90 for biological products.

[10] See 21 CFR 314.600 for drugs and 21 CFR 601.90 for biological products.

that FDA will rely on evidence from animal studies to provide substantial evidence[11] of effectiveness only when all of the following four criteria are met:

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.[12]

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

The use of the Animal Rule as a regulatory pathway to approval is intended for drugs developed to ameliorate or prevent serious or life-threatening conditions caused by chemical, biological, radiological, or nuclear substances regardless of whether the substances are considered potential threat agents for deliberate exposure (e.g., nerve agent, *Bacillus anthracis*) or threats to individuals' health from accidental exposure (e.g., emerging infectious pathogens, snake venom, industrial chemicals), provided that human efficacy studies are not ethical and field trials to study effectiveness of the drug are not feasible.

FDA will determine whether the previously noted criteria have been met and the Animal Rule can be used. In general, the determination of whether it is ethical to conduct deliberate exposure studies in humans is not difficult; however, the determination that human field trials are not feasible may be challenging. The feasibility issues to be considered will depend on the disease or condition to be studied and may change over time. For example, there may be circumstances that affect the feasibility of planning and conducting human field trials for the disease or condition, such as (1) a low prevalence and/or incidence, (2) an unpredictable incidence rate from year to year, (3) an inability to predict geographic locations where outbreaks may occur, (4) occurrences limited to areas lacking critical infrastructure, and/or (5) occurrences limited to areas

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[11] The term *substantial evidence* has been defined previously in section 505(d) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 355(d)) as follows: " . . . evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof."

[12] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

in which there is some extraordinary threat to subject or investigator safety. However, the international collaboration in response to the 2014–2015 Ebola epidemic in West Africa highlights the fact that determination of infeasibility of clinical trials can change over time. Sponsors should provide FDA with a clear rationale to support the use of the Animal Rule for the development of their drug before proceeding with drug development.

With regard to establishing evidence of efficacy, the Animal Rule states: "In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency."[13] Although data from different types of studies (e.g., in vitro studies, other types of animal studies, human studies) may be supportive, adequate and well-controlled animal efficacy studies are required for approval under the Animal Rule.

Supportive human data can include clinical efficacy data either from available human data for the same indication (as was the case for the 2006 approval under the Animal Rule of Cyanokit (hydroxocobalamin) for the treatment of known or suspected cyanide poisoning), or from available human data for a relevant non-Animal Rule-based indication (as was the case for the 2012 approval under the Animal Rule of Levaquin (levofloxacin) for the treatment of pneumonic and septicemic plague caused by *Yersinia pestis)*.

For Cyanokit (hydroxocobalamin), efficacy was established in a dog model of cyanide poisoning that FDA considered sufficiently well-characterized for predicting the response in humans. Existing human data from open-label, uncontrolled studies using hydroxocobalamin to treat cyanide poisoning from smoke inhalation, cyanide ingestion, or cyanide inhalation provided additional support for its approval.

For Levaquin (levofloxacin), efficacy was established in an African green monkey model of pneumonic plague that FDA considered sufficiently well-characterized for predicting the response in humans. Existing human data from its prior approvals for other respiratory infections (i.e., nosocomial and community-acquired pneumonias) provided additional support for its likely effectiveness in the treatment of pneumonic and septicemic plague.

When human efficacy data from a relevant indication may support the approval of the Animal Rule-based indication, FDA encourages sponsors to evaluate the drug in an indication for which obtaining human data is possible under a traditional regulatory pathway.[14] In addition to the previously discussed Levaquin (levofloxacin) example, another example of when human efficacy data obtained in a related human disease or condition may support the determination of efficacy for the Animal Rule-based indication is when the drug targets a pathway in the pathophysiological cascade that is common to both the disease or condition intended for evaluation under the Animal Rule and a disease or condition for which clinical trials are ethical and feasible.

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[13] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

[14] As stated in the preamble to the final rule "New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible" (the final rule) (67 FR 37988 at 37990 footnote 2, May 31, 2002), " . . . with anti-infective drug products, it would usually be expected that human data on safety and effectiveness for other indications may be available."

The Animal Rule specifies that the choice of species for the adequate and well-controlled efficacy studies must be appropriate with regard to the disease or condition of interest and the investigational drug.[15] There is no requirement for the use of a specific species. For each animal species selected by sponsors, the sponsors should provide scientific justification that the animal species exhibits key characteristics of the human disease or condition when the animal is exposed to the challenge agent.[16] In addition, the species should be selected based on an understanding of the drug's mechanism of action, such that the drug's effect in the animal species is expected to be predictive of its effect in humans, and to allow extrapolation from the animal data to the selection of an effective dose and regimen for humans.

The number of animal species necessary to support approval of a drug under the Animal Rule depends on the nature and clinical significance of any differences between the animal models[17] and humans with regard to the essential elements as described in section V. Sponsors should provide data or information to demonstrate that each animal model reflects key aspects of the pathophysiology of the human disease or condition of interest and that the response to the investigational drug in each animal model is likely to predict the response in humans.

FDA will evaluate the suitability of a proposed animal model on a case-by-case basis. Generally, the efficacy of the drug should be demonstrated in more than one animal species expected to react with a response predictive for humans. In certain circumstances, studies in more than two species may be necessary to model the relevant aspects of the human disease or condition and response to the investigational drug. If the effect is demonstrated in a single species that represents a sufficiently well-characterized animal model[18] for predicting the response in humans, then the Animal Rule allows for approval based on substantial evidence of effectiveness demonstrated in studies conducted in that species. The acceptability of using a single animal species will require FDA review and agreement on the body of evidence supporting the adequacy of the model. As discussed in the preamble to the final rule:[19]

> [The] 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

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[15] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

[16] As used in this guidance, the term *challenge agent* refers to the substance used to cause the disease or condition in the animal studies, whereas the term *etiologic agent* refers to the substance causing the disease or condition in humans.

[17] For the purpose of this guidance, an *animal model* is defined as a specific combination of an animal species, challenge agent, and route of exposure that produces a disease process or pathological condition that in multiple important aspects corresponds to the human disease or condition of interest.

[18] In the preamble to the final rule (67 FR 37988 at 37989, May 31, 2002), a *well-characterized animal model* was defined as "meaning the model has been adequately evaluated for its responsiveness."

[19] See 67 FR 37988 at 37991, May 31, 2002.

unnecessary. In addition, other human data for the product could provide support for such approvals.

When efficacy is demonstrated in a single study conducted in a sufficiently well-characterized animal model, it may be necessary to conduct a confirmatory efficacy study in that animal model.[20] The confirmatory study ideally should be conducted at a different laboratory, however, use of the same laboratory may be acceptable with justification. Supportive human efficacy data may negate the need for a confirmatory study, as was the case for Levaquin (levofloxacin) for pneumonic plague and Cyanokit (hydroxocobalamin) for cyanide poisoning.

There may be situations in which the application of the Animal Rule requires a more complex development plan. For example, variola virus (the etiologic agent of smallpox) presents a unique challenge because humans are the only known natural host, no animal species has been found to have comparable susceptibility to variola virus, and naturally occurring smallpox has been eradicated. Therefore, efficacy of investigational drugs developed to treat smallpox needs to be studied using other orthopoxviruses in relevant animal species (e.g., monkeypox in nonhuman primates, rabbitpox in rabbits, ectromelia in mice). Depending on the strength of the animal studies and other supporting evidence, the efficacy findings from such studies may support approval of the drug against variola. As with all animal efficacy studies, FDA strongly recommends that in such situations, sponsors discuss the scientific approach under consideration with the review division before initiating the animal studies.

Approval of a drug under the Animal Rule imposes three additional requirements, which are summarized below (for greater detail, see 21 CFR 314.610(b)(1) through (3) for drugs and 21 CFR 601.91(b)(1) through (3) for biological products):

1. Postmarketing studies (e.g., field studies) to provide evaluation of safety and clinical benefit if circumstances arise in which a study would be feasible and ethical (i.e., in the event an emergency arises and the drug is used). A plan or approach to conducting such a study must be included with the new drug application (NDA) or biologics license application (BLA).

2. Restrictions to ensure safe use, if needed (e.g., restricting distribution to facilities or health care practitioners with special training, requiring specified types of follow up, or imposing record keeping requirements).

3. Information to be provided in the labeling to patient recipients that explains that for ethical or feasibility reasons, the drug's approval was based on efficacy studies conducted in animals alone. This drug labeling should also include all the other relevant information required by FDA at the time of approval (e.g., directions for use, contraindications, a description of any reasonably foreseeable risks, adverse reactions,

[20] As stated in the preamble to the final rule, " . . . the animal studies should be replicated or substantiated in each species as needed to ensure credible results . . . " (67 FR 37988 at 37991, May 31, 2002).

anticipated benefits, and drug interactions). This information must be provided before administration or dispensing, if possible.

Products approved under the Animal Rule are subject to postmarketing recordkeeping and safety reporting applicable to all approved drug and biological products.[21] Information on withdrawal procedures, submission of promotional materials, and termination of certain requirements for products approved under the Animal Rule is specified in the regulations.[22]

III. REGULATORY CONSIDERATIONS

A. Drug Development Plan

Obtaining the body of evidence necessary to support approval of a drug using the Animal Rule is a complex and iterative process. FDA strongly encourages sponsors to establish early and ongoing communications with the Agency. Sponsors also may wish to seek input from public health officials and/or the military about the potential need for, and operational use of, the investigational drug and discuss this with FDA. Developing a drug development plan will support the discussion of important issues, including, but not limited to, the following:

- The proposed indication and whether a drug can be developed under the Animal Rule
- The design of an animal study as it relates to the anticipated clinical use of the drug during an incident
- The development and/or selection of the animal models, including, when necessary, the design of the natural history studies
- The results of the proof-of-concept studies
- The proposed methods for selecting an effective dose and regimen in humans
- The design of the adequate and well-controlled animal efficacy studies intended to provide the primary evidence of effectiveness of the drug
- The proposed approach for ensuring the quality and integrity of data[23]
- The size and composition of the human safety database

[21] See 21 CFR 314.630 for drugs and 21 CFR 601.93 for biological products.

[22] See 21 CFR 314.620, 21 CFR 314.640, and 21 CFR 314.650, respectively, for drugs and 21 CFR 601.92, 21 CFR 601.94, and 21 CFR 601.95, respectively, for biological products.

[23] In issuing the Animal Rule, FDA stated that " . . . studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices (21 CFR part 58) regulations . . . " (67 FR 37988 at 37989, May 31, 2002). The good laboratory practice (GLP) regulations, however, were developed as a quality system for nonclinical safety studies. FDA's current expectations are described in section IV.B.

- Plans or approaches for conducting the required postmarketing studies (e.g., field studies) to demonstrate safety and clinical benefit when such studies are feasible and ethical[24]
- Timelines and/or milestones for FDA feedback or meetings
- Eligibility for expedited development and review designation programs
- Additional issues critical to the sponsor's funding agencies[25]

Drug development is data-driven; any development plan should allow for modification or refinement as data are gathered and analyzed and projections or expectations change. It is the sponsor's responsibility to provide complete and accurate submissions. Sponsors should explain any proposed deviations from the recommendations expressed in this guidance. The potential impact of these deviations on the drug development program should be discussed with FDA before the conduct of the relevant studies.

FDA strongly recommends that sponsors obtain Agency concurrence on the design of the adequate and well-controlled animal efficacy studies because these substitute for the efficacy trials in humans (see sections VI and X). Sponsors should allow adequate time for FDA review, comment, and agreement before initiating these studies to ensure that the study design is adequate to support the proposed indication.

The protocols for animal efficacy studies intended to provide primary evidence of effectiveness are eligible for evaluation under special protocol assessment (SPA) provisions.[26,27] Before submitting a Request for SPA, the sponsor should have FDA concurrence on the model proposed for use in the efficacy study (including, but not limited to, the species, the details of the challenge agent, the conditions of exposure) and the method that will be used to extrapolate from the animal data to select an effective dose and regimen in humans.

Drugs developed under the Animal Rule may be eligible for two of the expedited development and review programs[28] (fast track and priority review) or other FDA programs, such as orphan drug designation.[29] Sponsors requesting these designations should use established procedures. Drugs being developed under the Animal Rule do not meet the statutory requirement for

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[24] See 21 CFR 314.610(b)(1) for drugs and 21 CFR 601.91(b)(1) for biological products.

[25] Product development plans required by funding agencies for medical countermeasures against CBRN agents may dictate certain proof-of-concept studies and an accelerated timeline for efficacy studies in animals. The sponsor's relationship with its funding agency is independent of its relationship with FDA.

[26] Section 505(b)(5)(B) of the FD&C Act (as amended by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013, Public Law 113-5) provides for the use of SPA provisions "in the case where human efficacy studies are not ethical or feasible, of animal and any associated clinical trials which, in combination, are intended to form the primary basis of an effectiveness claim."

[27] For procedural information, see FDA's guidance for industry *Special Protocol Assessment*.

[28] See FDA's guidance for industry *Expedited Programs for Serious Conditions–Drugs and Biologics*.

[29] For information on orphan drug designation, see the Web page, available at http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/HowtoapplyforOrphanProductDesignation/ucm135122.htm.

breakthrough therapy designation, which specifically requires clinical evidence of a treatment effect.[30] The Best Pharmaceuticals for Children Act (BPCA)[31] and the Pediatric Research Equity Act of 2003 (PREA)[32] may also apply to drugs developed under the Animal Rule.

Sponsors should note that FDA may seek input from advisory committees for various issues related to the Animal Rule. Issues for discussion can include whether the Animal Rule is the appropriate regulatory development pathway for drugs intended for a specific indication, concurrence on the animal model of a disease or condition, the acceptability of the use of an animal model with a specific investigational drug, the design of adequate and well-controlled animal efficacy studies, and whether the data obtained support approval. In some instances, more than one advisory committee meeting may be warranted at different times in a single development program.

B. Access to Investigational Drugs During a Public Health Emergency

Data collected from animal efficacy studies may support the emergency use of drugs under an investigational new drug application (IND)[33] or an emergency use authorization (EUA).[34] FDA's decision to allow emergency use of a drug under an IND or EUA will be made on a case-by-case basis, taking into account the anticipated or actual emergency, size of the affected population, data included in the submission, and risk-benefit analysis. A decision to allow emergency use of the drug under an IND or EUA, based on data submitted in support of either mechanism, should not be viewed as an FDA determination on approvability of the drug or a final drug development goal. FDA emphasizes that drug development and systematic data collection should be continued to obtain the body of evidence to support drug approval and associated postmarketing requirements. In the event of an unexpected increase or occurrence of

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[30] See section 506 of the FD&C Act (21 U.S.C. 356) (as amended by the Food and Drug Administration Safety and Innovation Act (FDASIA), Public Law 112-144).

[31] See Public Law 107-109.

[32] See Public Law 108-155.

[33] Expanded access for individual patients (including for emergency use), intermediate-size patient populations, and large patient populations (under a treatment IND or treatment protocol) are described in 21 CFR 312.300 through 312.320. Individual patient INDs, however, are not a feasible strategy for a large-scale event requiring mass access to an investigational drug. Sponsors anticipating multiple access requests for an investigational drug should discuss proposals for IND protocols with FDA, including any potential for clinical trial design that could provide safety and efficacy information as well as access. FDA has issued two draft guidances on expanded access. When the guidance on *Expanded Access to Investigational Drugs for Treatment Use – Qs & As* and the guidance on *Individual Patient Expanded Access Applications: Form FDA 3926* are finalized, they will represent the Agency's current thinking on this topic. Additional information on expanded access can be found on FDA's Expanded Access (Compassionate Use) Web page, available at http://www.fda.gov/NewsEvents/PublicHealthFocus/ExpandedAccessCompassionateUse/default.htm.

[34] EUA criteria are described in FDA's guidance *Emergency Use Authorization of Medical Products.* Additional information on EUA can be found on FDA's Emergency Use Authorization Web page, available at http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm182568.htm.

a disease or condition, sponsors should discuss with FDA if field studies have become feasible and ethical (see related discussion in section II).

C. Communications With FDA

Sponsors are encouraged to hold discussions with FDA in the early stages of a drug development program. Sponsors unsure of the appropriate regulatory review division or office for their investigational drugs can inquire through the electronic mailbox, CDER-CBER-ARJurisdiction@fda.hhs.gov, provided by CDER and CBER for this sole purpose.

Sponsors should consult Agency guidance regarding the process and expectations for formal meetings.[35] The sponsor and review division should discuss the avenues and expectations for obtaining Agency concurrence on the design of the adequate and well-controlled animal efficacy studies and for addressing extenuating or unforeseen circumstances. It is the sponsor's responsibility to build sufficient time into the development plan to permit the review, discussion, and resolution of issues prior to the initiation of relevant studies. FDA will try to accommodate the sponsor should unforeseen circumstances arise.

Some of the drug development issues that should be the subject of meetings with FDA[36] will differ from those for drugs developed under other regulatory pathways. Examples of issues for Animal Rule drug development discussions are listed in section III.A.

D. Animal Model Qualification Program

The Animal Model Qualification Program (AMQP)[37] is jointly supported by CDER and CBER to address the need for publicly available animal models for use in drug development under the

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[35] See FDA's guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants* (Revision 1). In the *Federal Register* of March 11, 2015 (80 FR 12822), FDA published a notice announcing the availability of a draft guidance *Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products* (Revision 2). The revised draft guidance updates the guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants* (Revision 1) and, when finalized, will represent the Agency's current thinking on the topic.

[36] Section 565(d) of the FD&C Act (21 U.S.C. 360bbb-4(d)) (as amended by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013, Public Law 113-5) provides that sponsors developing countermeasures under the Animal Rule may request and receive two meetings with FDA, one to discuss "proposed animal model development activities" and the other "prior to initiating pivotal animal studies." These meetings and procedures for obtaining such meetings are within the scope of FDA's guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants* and satisfy this requirement (see the preceding footnote).

[37] The AMQP was established under FDA's Drug Development Tools (DDT) Qualification Programs (see FDA's guidance for industry and FDA staff *Qualification Process for Drug Development Tools*). Additional information about qualifying animal models can be accessed through the Animal Model Qualification Program Web page, available at http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm284078.htm.

Animal Rule.[38] Through this program, animal models are evaluated and qualified for a specific context of use (COU) that describes the appropriate use and application of the qualified animal model in drug development and regulatory review and specifies the details[39] necessary to replicate the model. Submitting a model for qualification is voluntary. Approval under the Animal Rule does not require the use of a qualified model.

Qualification is a regulatory conclusion[40,41] that is not linked to a specific drug. Qualification of an animal model through the AMQP indicates that (1) FDA has concluded that a specific animal species, given a specific challenge agent by a specific route, produces a disease process or condition that in multiple important aspects corresponds to the human disease or condition of interest, and (2) FDA has accepted the description of the model's appropriate use in regulatory applications, including the definition of the parameters of the disease or condition that will be used as measures of quality control and quality assurance when the model is used. After the animal model is qualified, FDA does not have to reevaluate this conclusion each time this qualified model is used within the bounds of its stated COU.

Before using a qualified animal model of a disease or condition in an adequate and well-controlled efficacy study, the sponsor of an investigational drug should establish that the model is a suitable test system for the drug with regard to the drug's mechanism of action and related host factors and the ability to extrapolate from the animal data to select a dose and regimen in humans (see section V.B). Similarly, because animal models are qualified without reference to a specific drug, the use of a qualified animal model does not ensure that the model will be found acceptable as "a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans," as stated in the second criterion for drug approval under the Animal Rule.[42] FDA may not accept evidence of effectiveness from a single animal model (even if it is qualified) for an investigational drug unless FDA concludes there is sufficient evidence that the results generated in this model adequately predict the response to the drug in humans. The regulatory decision to allow approval of a drug based on the use of an animal model in a single species will be made by the review division on a case-by-case basis (see section II).

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[38] Qualification of an animal model is voluntary and is limited to animal models developed for the intended purpose of supporting the development programs for multiple investigational drugs for the same targeted disease or condition. A model developed by a sponsor of an investigational drug for the intended purpose of use in the development program of that drug alone will not be eligible for qualification.

[39] These details include, but are not limited to, the following: characterization of the animals to be used, characterization and preparation of the challenge agent, procedural information for the challenge agent exposure, identification of the primary and any secondary efficacy endpoints, triggers for intervention, and ranges of values of key parameters of the disease or condition that will be used as measures of quality control and quality assurance when the model is replicated.

[40] Woodcock, J, S Buckman, F Goodsaid, MK Walton, I Zineh, 2011, Qualifying Biomarkers for Use in Drug Development: A US Food and Drug Administration Overview, *Expert Opin Med Diagn*, 5(5):369-374.

[41] The qualification recommendation for the animal model and its COU will be made publicly available and can be referenced by its FDA-assigned tracking number for use in regulatory submissions.

[42] See 21 CFR 314.610(a)(2) for drugs and 21 CFR 601.91(a)(2) for biological products.

Qualification is a regulatory conclusion; therefore, FDA recommends the use of the GLP regulations, to the extent practicable, for the model-defining natural history studies[43] submitted to support the qualification of an animal model. This will facilitate conduct of the study in a manner that ensures data quality and integrity. These model-defining natural history studies will be subject to inspection by FDA to verify the quality and integrity of the data (see section IV.B).

IV. ANIMAL STUDIES – GENERAL EXPECTATIONS

The general expectations discussed in this section apply to the Animal Rule-specific studies, i.e., the natural history studies that define the animal model in which efficacy will be tested, the adequate and well-controlled animal efficacy studies intended to provide the primary evidence of effectiveness to support approval,[44] and the PK and/or pharmacodynamic (PD) studies in animals used to select a dose and regimen in humans. Specific recommendations for natural history studies are discussed in Appendix C. Specific recommendations on design considerations for the adequate and well-controlled efficacy studies in animals are discussed in section VI and section X, with additional information for preventive vaccines and cellular and gene therapies in section VII. Specific recommendations for the PK and/or PD studies used to select a dose and regimen for humans are discussed in section V.B.2.

A. Animals Used in Investigations

For the Animal Rule-specific studies, the animals should be research naïve. Any prior research experience, even as a control animal, has the potential to cause stress and alter an animal's physiological responses. The number of animals should be determined to ensure scientifically valid results. Well-designed experiments use a sufficient number of animals to achieve the scientific objective, include the necessary control groups, and incorporate appropriate statistical analyses. FDA expects adequate representation of both sexes in these studies. The male/female composition of the study groups should be justified.

Appropriately designed protocols generally control for age, body weight, current health status, and the physical environment of the test animals. When defining the animal characteristics for the development of animal models and the subsequent efficacy studies using those models, sponsors should discuss with FDA the applicability of the animal models and the results obtained from the efficacy studies to the diverse human population.

Appropriate inclusion and exclusion criteria for the acceptance of the animals into the study should be prespecified and discussed with FDA before initiating the studies. The information that should be provided for the characterization of individual animals used in the investigation is described in section IV.D.

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[43] In the context of animal model qualification, the *model-defining natural history studies* are the animal studies that establish the ranges of values of key parameters of the disease or condition that will be specified in the COU for the qualified model and that will be used as measures of quality control and quality assurance when the model is replicated.

[44] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

B. Study Conduct

The adequate and well-controlled animal efficacy studies and the PK and/or PD studies in animals used to select a dose and regimen in humans should be conducted in a manner that ensures data quality and integrity, as would be expected for the studies in humans submitted to establish effectiveness and any other studies needed to support the labeling of a drug approved under a traditional regulatory pathway. There are no regulations that specifically address data quality and integrity issues for Animal Rule-specific studies.[45] The GLP regulations[46] were developed as a quality system for nonclinical safety studies. Nonetheless, these regulations provide a framework (e.g., definitions, procedures, roles and responsibilities, and controls) for the conduct of nonclinical studies, and FDA considers them to be a well-established and relevant system for ensuring data quality and integrity for the adequate and well-controlled animal efficacy studies and the PK and/or PD studies in animals used to select a dose and regimen in humans. FDA, therefore, recommends the use of the GLP regulations, to the extent practicable, to ensure the quality and integrity of data from these studies.[47]

There may be justifiable limitations in the ability to apply the GLP regulations when conducting these studies, especially for those using challenge agents that require high containment facilities. Before initiating these studies, sponsors should identify aspects of the studies anticipated to be a challenge with regard to the GLP regulations and propose methods for adapting the studies to ensure the quality and integrity of the resulting data. Sponsors should seek concurrence from FDA on the data quality and integrity plan before the studies are initiated.

The adequate and well-controlled animal efficacy studies and the PK and/or PD studies in animals used to select a dose and regimen in humans serve as the basis for a regulatory action (e.g., approval) under the Animal Rule. Thus, FDA has the authority to inspect these studies prior to taking an action. Inspections will be conducted to verify the quality and integrity of the raw data, supporting documentation, facilities, equipment, and the results submitted to FDA in the final report. *Quality* includes, but is not limited to, whether the study was conducted in accordance with the protocol, standard operating procedures, and applicable standards of research. *Integrity* includes, but is not limited to, the assurance that the raw data and documentation are consistent with reported results. FDA will verify that study personnel followed the agreed-upon data quality and integrity plan. Inspectional observations will be shared with the inspected entity and evaluated by the review division to determine the impact of the observations on the acceptability of the data to support drug approval.

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[45] In issuing the Animal Rule, FDA stated that " . . . studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices (21 CFR part 58) regulations . . . " (67 FR 37988 at 37989, May 31, 2002). The good laboratory practice (GLP) regulations, however, were developed as a quality system for nonclinical safety studies. This section describes FDA's current expectations.

[46] See 21 CFR part 58.

[47] In addition, FDA recommends the use of the GLP regulations, to the extent practicable, for the *model-defining natural history studies* submitted to support the qualification of an animal model (see section III.D). Qualification is a regulatory conclusion, and thus, these studies should be conducted in a manner that ensures data quality and integrity.

Animal studies must comply with applicable laws and regulations as prescribed by the Animal Welfare Act[48] and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.[49] All studies should comply with general principles for the care and use of animals in biomedical research (see Appendix A). Sponsors should ensure that adequate safety and security provisions are in place for all studies when needed. For example, for select agents and toxins, sponsors must adhere to the regulations found under 42 CFR part 73, and when applicable, sponsors should comply with standards on the use of biosafety level (BSL) laboratory facilities.[50]

The investigational drug used in the adequate and well-controlled animal efficacy studies and the animal PK and/or PD studies used to select a dose and regimen in humans ideally should be manufactured under current good manufacturing practice regulations.[51] The investigational drug also should be as close as practicable to the to-be-marketed drug; any differences should be discussed with the review division before studies are initiated.

C. Types of Animal Care Interventions

As used in this guidance, animal care interventions in animal studies are divided into three categories based on the rationale for their use: (1) intervention as part of adequate veterinary care, (2) intervention as supportive care to mimic the human clinical scenario, and (3) intervention to permit the manifestation of the disease or condition of interest for the purpose of model development. These categories of interventions are discussed individually in Appendix B. The potential effects of the interventions on the animal (e.g., toxicity, effects on the immune system) and on the pharmacokinetics, pharmacodynamics, and efficacy of the investigational drug should be considered in the design and interpretation of each study. In addition, protocols for the adequate and well-controlled efficacy studies should include plans for addressing the impact of potential differences in care among animals.

D. The Study Report

FDA expects that complete, final study reports will be submitted for the Animal Rule-specific studies. Complete study reports should include, but are not limited to, the following:

- The prospectively designed protocol (including the statistical analysis plan), all protocol amendments, and a description of all protocol deviations

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[48] See 7 U.S.C. 2131 et seq.

[49] U.S. Department of Health and Human Services, National Institutes of Health, Office of Laboratory Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Revised 2015, available at http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on October 16, 2015.

[50] U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, 2010, *Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition, Atlanta, GA: CDC.

[51] See 21 CFR parts 210 and 211.

- Detailed descriptions of the elements of the study design, including the characterization of the animals used in the study;[52] information on the formulations and administration of the investigational drug and controls; and information on the characterization, preparation, and delivery of the challenge agent
- A comprehensive description of study procedures
- The results[53] of each parameter or variable evaluated at each time point in the study and any unscheduled medical intervention
- The final audited[54] study report that includes analyses and interpretation of the study data and explanation of any deviations from the agreed-upon plan for data quality and integrity

Preliminary plans for collection, organization, format, and level of detail of study data should be discussed with the review division before conducting these studies. Sponsors are encouraged to submit prototype versions of the study datasets prior to finalization of datasets.

E. Submission of the Study Report and Data

The submission of study data in a standardized electronic format supports data analysis and review and has therefore been encouraged by FDA. Section 1136 of FDASIA[55] requires that nonclinical and clinical study data be submitted electronically to FDA in a format specified by guidance. FDA's guidance for industry *Providing Regulatory Submissions in Electronic Format — Standardized Study Data*[56] specifies that electronic submissions must be in a format that FDA can process, review, and archive and describes the requirements, including the types of

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[52] The individual animal information should include, when appropriate, species; strains and substrains (when applicable); breed (when applicable); age; sex; body weight; vendor source; origin of the animal (to the extent known); procedures for identification and individual animal identification; physiological status (e.g., adult, juvenile, lactating, and pregnant); data collected during routine husbandry pre- and post-protocol assignment, including pre-study health screen, health records, and medications or therapies administered; and an adequate description of housing and husbandry conditions. For individual animal tracking purposes, a table that cross-references the unique animal identification number for the study, treatment allocation, fate or disposition, and chain of custody should be submitted. For each animal assigned more than one identification number during life, the table also should include reference to all other identification numbers (e.g., a unique animal number assigned by the source).

[53] These results should include group summary tabulations, line listings of the results for each individual animal, copies of the individual animal case report forms (all veterinary medical records), and any other primary data necessary for the reconstruction of key analyses and evaluation of the study report.

[54] Final study reports should be audited for quality assurance in accordance with the data quality and integrity plan to verify that the protocol, protocol amendments, and standard operating procedures were followed by study personnel; the raw data were accurately recorded and support the findings and conclusions in study reports; and any findings identified during the audit as having the potential to affect the quality and integrity of the data have been addressed.

[55] See Public Law 112-144.

[56] This guidance implements the electronic submission requirements of section 745A(a) of the FD&C Act (21 U.S.C. 379k-1(a) (added by section 1136 of FDASIA, Public Law 112-144).

submissions to which these requirements apply[57] and timetables for the implementation. For studies initiated prior to the requirement date specified in the timetables, sponsors are strongly encouraged to submit study data in a standardized electronic format. Sponsors should consider the implementation of data standards and seek FDA feedback as early as possible during animal model and drug development, so that the data standards are included in the design, conduct, and analysis of studies.[58]

The Electronic Common Technical Document (eCTD) is the standard format for regulatory submissions to CBER and CDER.[59] The eCTD does not provide a specific location for the natural history or model characterization studies conducted in animals and for the adequate and well-controlled animal efficacy studies. For consistency, it is recommended that these studies be submitted to Module 4 (Nonclinical Study Reports), section 4.2.1.1 (Primary Pharmacodynamics). This recommendation does not determine the disciplines of the primary reviewers for the studies; that decision is the purview of the FDA review division.

V. ESSENTIAL ELEMENTS OF AN ANIMAL MODEL

The selection of an animal model for an efficacy study should be based on its adequacy as a model of key elements of the human disease or condition and its suitability with regard to the investigational drug. Section V.A describes the elements related to the disease or condition induced by the etiologic or challenge agent.[60] It is the sponsor's responsibility to provide, to the fullest extent possible, a documented summary of the etiologic agent-induced human disease or condition and a detailed discussion that delineates how these data support selection of the proposed animal model. Evidence supporting the relevance of an animal model to a human disease or condition can be obtained from various sources[61] that provide adequate documentation of study quality.[62] For example, data from literature or historical studies may support the use of an animal model when the reports include a level of detail that is sufficient to assess the appropriateness of the animal model. The source, organization, format, and level of detail of the available study data should be discussed with the review division before submitting the data.

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[57] An IND for a drug or biological product intended to progress to an NDA or BLA, respectively, is considered to be a commercial IND and, therefore, is subject to these requirements.

[58] Information is available through FDA's Web page, Study Data Standards Resources, available at http://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/default.htm.

[59] See FDA's guidance for industry *Providing Regulatory Submissions in Electronic Format – Certain Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications* (Revision 3).

[60] As used in this guidance, the term *etiologic agent* refers to the substance causing the disease or condition in humans. The term *challenge agent* refers to the substance used to cause the disease or condition in the animal studies.

[61] Comparable to the sources of clinical data described in 21 CFR 314.50(d)(5)(iv).

[62] Comparable to the discussion of the documentation of the quality of evidence described in FDA's guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products.*

Section V.B describes elements related to the investigational drug and the selection of an effective dose in humans. The sponsor should provide justification of the suitability of each model based on the investigational drug's mechanism of action, dosage form, and route of administration, and the method proposed to extrapolate from the animal data to select a dose and regimen in humans. Issues related to animal model development for one or more investigational drugs that are to be developed for use in combination or concurrently are beyond the scope of this guidance and should be discussed with the review division.

The following essential elements should be considered in the development and/or the selection of an animal model.[63] Any element not achievable for an etiologic or challenge agent or drug under investigation should be discussed with FDA.

A. Elements Related to the Etiologic or Challenge Agent-Induced Disease or Condition

1. Characteristics of the Etiologic or Challenge Agent That Influence the Disease or Condition

The characteristics of the specific etiologic or challenge agent that influence the disease or condition under study include its pathophysiological mechanisms of toxicity or virulence, the route of exposure, and the dose and quantification of exposure. These characteristics are discussed below.

a. The Challenge Agent

The challenge agent used to establish the disease or condition in the animal studies generally should be the same as the etiologic agent that causes the human disease or condition. If the challenge agent is different from the etiologic agent, the sponsor should provide justification for the use of that challenge agent. The sponsor also should explain why, when used in the proposed animal model, the challenge agent should be considered suitable for establishing effectiveness of the investigational drug in humans against the intended etiologic agent. For example, for an animal efficacy study to support approval of a drug to treat the gastrointestinal subsyndrome of acute radiation syndrome (GI-ARS), 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 and dose of radiation used in the study and their relevance to the clinical situation.

The selection of a biological challenge agent should be based on known virulence factors, using standardized, validated test methods, and the challenge agent used ideally should be of low passage history. For plague studies conducted in animals, pigmented *Y. pestis* strains are preferred because non-pigmented strains

[63] See section IX for the associated Checklist of Essential Elements of an Animal Model.

rarely cause disease. Generally, bacterial and viral strains known to be associated with outbreaks of human disease should be used for the natural history and animal efficacy studies (e.g., Ebola Zaire virus isolated from a human who died from an infection during an outbreak should be used in the animal studies); however, there may be issues regarding differences in the strain or serotype of the biological agent that will limit the relevance, or preclude the use, of data obtained to support the proposed clinical indication. For example, there may be various strains of a bacterium that differ in the expression of virulence factors. When an investigational drug targets a particular virulence factor or pathogenic mechanism associated with a particular virulence factor, effectiveness may be limited to strains that express that particular virulence factor, and an indication for all variants of that bacterium may not be warranted.

The challenge agents and their preparations should be characterized in terms relevant for their category (i.e., biological, chemical, radiological, or nuclear). For biological agents, these terms should include passage history, method of preparation, and concentration. For chemical agents, characteristics should include source and stated purity of the agent, dosing formulation, concentration, and stability under the conditions of use. For radiation or nuclear challenges, the terms should include the type and source of radiation. Such characterization facilitates comparison among studies.

b. Pathophysiological Mechanisms of Toxicity or Virulence

The pathophysiological mechanisms of toxicity or virulence of the challenge agent expressed in the animal model should be similar to those expressed by the etiologic agent in humans. For a biological agent, the pathophysiological mechanisms of virulence are the pathogenic determinants of the microbe (i.e., its genetic, biochemical, or structural features that enable it to elicit disease in a host). Examples of microbial pathogenic determinants include toxins, substances that promote invasion, substances that modulate inflammation, substances that cross-react with host tissues, and mechanisms to evade host defenses. For a chemical agent, the mechanisms of toxicity can include receptor binding, inhibition of enzymes, and binding of intracellular components. For radiation, the mechanisms of toxicity include DNA damage and the generation of free radicals.

c. Route of Exposure

When the pathogenesis of the disease or condition is dependent on the route of exposure to the challenge agent, the animal models should use the same route as that anticipated in humans. For example, human infection with *Y. pestis* can occur through flea bite or inhalational exposure. Exposure through a flea bite typically leads to development of bubonic plague, which can progress to septicemic plague, whereas inhalational exposure usually leads to development of pneumonic and septicemic plague. Thus, an animal model of pneumonic plague should use an inhalational route of exposure to *Y. pestis*.

In cases when the challenge agent-induced disease or condition is not clearly tied to its route of exposure, alternate routes of exposure may be acceptable. If a sponsor proposes to use a route of exposure to the challenge agent in animals that is different from that expected in humans, scientific justification should be provided. If such an approach is under consideration, it should be discussed with FDA before initiation of the natural history and animal efficacy studies.

Sponsors should discuss potential paths forward with FDA when trying to develop a drug for a disease or condition for which limited or no human data are available for the etiologic agent by the route of exposure in the proposed clinical indication.

d. Dose and Quantification of Exposure

Ideally, the sponsor should use a challenge agent dose that produces a disease or condition in animals that corresponds to the expected extent and severity of the human disease or condition. The dose of the etiologic agent that causes the human disease or condition may not be known, or the exposure may not be fully quantifiable, as can be the case for the radiation exposure to humans following a nuclear incident. In such a case, a sponsor developing a drug to treat the hematopoietic subsyndrome of acute radiation syndrome (H-ARS) should provide a detailed description of the methods of radiation exposure used in the animal studies, including type and source of radiation, dose and dose rate, whole versus partial body irradiation, and their relevance to the clinical situation.

The method for the delivery of the challenge agent should be described in sufficient detail to permit replication of test conditions. Reliable quantification using a validated assay and reproducibility of the challenge agent dose should be demonstrated from model development through its use in the animal efficacy studies. In general, the target dose and actual dose delivered to an individual animal should be expressed in absolute terms (e.g., colony forming units or plaque forming units for a biological agent, or the radiation dose expressed in gray), as well as in terms that indicate the toxicity or virulence of the challenge agent (e.g., the LD_{50}, which is the dose sufficient to kill 50% of those exposed to the agent).

2. *Host Susceptibility and Response*

The animal species chosen for model development should be susceptible to the challenge agent. Also, if the host immune response is part of the pathogenesis of the disease or condition in humans, it should play a similar role in the animal model. FDA recognizes there may be susceptibility differences among species. For example, an animal species used to study the efficacy of a treatment for H-ARS may require a threshold of radiation exposure to develop the subsyndrome that is different from the threshold that is needed in humans. If the thresholds in humans and in the animal model differ greatly, the

suitability of the animal model may be called into question and the model should be discussed with FDA. The animal species may still be appropriate for study if the resulting disease or condition and the progression of the associated manifestations are similar in the animal species and humans. The factors that determine differences in susceptibility to the agent should be described to the extent possible. For example, when selecting an animal model to study the lethal effects of soman, an important consideration is the endogenous level of carboxylesterase in the selected animal species. This enzyme has a detoxifying effect on soman. Certain species are less susceptible to the effects of soman because of higher endogenous levels of this enzyme.[64]

Animal species that are not susceptible to the etiologic agent may not be suitable models for efficacy studies. Other approaches to the accrual of relevant animal data may need to be explored (for an example, see the discussion of the variola virus and human smallpox in section II).

The response to the challenge agent (i.e., the resulting disease or condition) manifested by the animal species should be similar to the disease or condition seen in humans exposed to the etiologic agent with respect to the proposed clinical indication. 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 will be acceptable to FDA for the development of a treatment for mustard gas-induced injury to the skin. Similarly, although mice are known to be susceptible to *B. anthracis*, the pathogenesis of the disease process in mice differs from that in humans. Therefore, mice may not be appropriate models for anthrax efficacy studies.[65] If the sponsor believes that such a model is supportive to the study of its investigational drug, a justification should be provided and the model should be discussed with FDA before proceeding.

3. Natural History of the Disease or Condition – Pathophysiological Comparability

The general expectations for the design and conduct of animal natural history studies are described in Appendix C. The natural history of the disease or condition in the selected animal species and in humans should be characterized and the similarities and differences compared and contrasted. This information should be discussed with FDA before

[64] Pretreatment with pyridostigmine bromide was shown to decrease the lethality of soman in rhesus macaques and guinea pigs. Pyridostigmine bromide's protective effect was not consistently demonstrated in other species tested because these other species were protected from soman by high levels of endogenous carboxylesterase, an enzyme that detoxifies soman. To confirm the theory for inter-species differences, a study was conducted in rats pretreated with a carboxylesterase inhibitor before exposure to soman. In these carboxylesterase inhibitor-treated rats, pretreatment with pyridostigmine bromide demonstrated decreased lethality to soman, compared to rats not pretreated with pyridostigmine bromide. These results were similar to the survival benefit demonstrated with pyridostigmine bromide in the rhesus macaques.

[65] Leffel, EK and MLM Pitt, "Characterization of New and Advancement of Existing Animal Models of *Bacillus anthracis* Infection," in JR Swearengen (ed.), *Biodefense Research Methodology and Animal Models*, Boca Raton, FL: CRC Press, 2012, pp. 81-98.

initiation of the efficacy studies. To facilitate these discussions, sponsors should provide an adequately documented summary of the etiologic agent-induced human disease or condition and a detailed discussion as to how these data support the selection of the animal model. This information should include (but not be limited to) the following parameters:

- Time from exposure to onset of the manifestations of disease or injury
- Time course and order of the progression of the manifestations
- Manifestations (e.g., signs and symptoms, clinical and pathological features, laboratory parameters, extent of organ involvement, morbidity, and outcome)

These parameters can be influenced by many factors, such as the type of etiologic or challenge agent, virulence or lethal potential of the etiologic or challenge agent, route of exposure, concentration, host factors (including immune status), and medical management in humans versus animal care interventions. Potential endpoints for evaluating efficacy should also be discussed. Experimental parameters may need to be modified to create a disease or condition that more closely mimics that seen in humans, or the model may need to be tailored for the proposed clinical indication.

It may not always be possible to compare the pathophysiology of the disease or condition in animal models to that in humans. For some diseases or conditions, relevant human data are not available, or the data are limited to references in the literature describing the end-stage pathology for symptomatic patients. For example, the description of the pathophysiology of H-ARS has been derived mainly from the literature discussing accidental occurrences in which humans received variable exposures to radiation.

a. Time to Onset

The time to onset of the disease or condition in animals should be reasonably similar to that in humans. Factors such as route of exposure, level of exposure (e.g., dose, concentration), and species or strain of the infective microorganism can influence time to onset and should be taken into consideration in model development.

b. Progression

Ideally, the progression of the manifestations of the disease or condition (including the order of their presentation) in the selected animal models should be similar to that observed in humans; when different, it should allow time for identification of the disease or condition, intervention, and assessment of the outcome of treatment. Demonstration of the effect of the investigational drug may be more challenging when the time between onset and death is short. For example, hamsters challenged with *B. anthracis* have such a rapid disease progression that this species is not useful for testing the efficacy of drugs for the

treatment of anthrax in humans. The route of exposure may affect the progression of the disease or condition, including the time course.

c. Manifestations

The manifestations of the disease or condition, including laboratory parameters, histopathology, gross pathology, and outcome (morbidity and/or mortality), and their known time courses should be compared between untreated animals and humans (e.g., historical information from human cases). Differences should be clearly noted and explained based on the understanding of the pathophysiological differences between the species, when possible. Certain manifestations in humans (e.g., fever, shortness of breath) may be difficult to discern in animals through clinical observation; therefore, a sponsor may need to use more refined techniques, such as telemetry, to evaluate affected animals. Animals in the natural history studies and the efficacy studies should be observed with greater frequency over the entire course of the day than would be typical of most animal studies used for toxicology evaluation. The frequency of observations per day may vary over the course of the study, depending on the animal species and strain, the experimental conditions, and the mechanism of disease or injury of the challenge agent. The observation frequency should be adequate to characterize the course of disease or condition and to define the desired treatment triggers and efficacy endpoints.

Animals should be evaluated in the context of prospectively defined euthanasia criteria to ensure animal welfare. Study results may be influenced by the euthanasia criteria used. Sample integrity may be compromised if the sample is not obtained prior to or immediately after death or euthanasia. Study personnel should be blinded to exposure status and should follow the observation frequency paradigm and euthanasia criteria to minimize the possibility of unnecessary suffering of moribund animals and to reduce potential study bias as much as possible.

4. Trigger for Intervention

A clearly defined trigger for intervention should be established for use in animal efficacy studies when needed (e.g., post-exposure prophylaxis and treatment indications). The trigger for intervention should be identified based on the natural history studies. For a post-exposure prophylaxis indication, a trigger for intervention should be defined to ensure drug administration within a reasonable time frame after exposure to the challenge agent and prior to the onset of the disease or condition of interest. The time frame should be justified with respect to administration of the drug to humans. Animals cannot simulate the health-seeking behavior manifested by humans; therefore, a clearly defined trigger for intervention for a treatment indication will ensure that treatment is not initiated until the disease or injury process is established. If signs observed in the animal model closely resemble those in humans and are predictive for the disease, they may serve as the trigger for intervention.

In the absence of disease- or condition-defining manifestations, sponsors can propose a biomarker as a trigger for intervention, if information can be provided that it relates to the pathophysiology of the disease or condition. The utility of the biomarker should be justified through an analysis that correlates the time course of the appearance of the parameter in animals with the onset of the disease or condition in the animals. The assay method and its performance characteristics for a biomarker used as a trigger for intervention in animal studies should be adequately described.

Sponsors are encouraged to initiate early discussions with FDA regarding the utility of the chosen triggers for intervention, particularly when the manifestations of the disease or condition in the animals differ from those in humans, or when a biomarker is used as a trigger for intervention.

B. Elements Related to the Investigational Drug and the Selection of an Effective Dose in Humans

The concepts discussed in this section apply primarily to small molecule drugs and therapeutic proteins. For information regarding preventive vaccines and cellular and gene therapies, consult sections VII.A and VII.B, respectively.

1. The Investigational Drug

The characterization of the investigational drug with regard to identity, concentration, purity, composition, and stability is the same under the Animal Rule as for any investigational drug developed under other regulatory pathways. Additional elements of the investigational drug that are important considerations for animal model selection include the mechanism of action, drug class, dosage form, and route of administration. These elements are discussed below.

a. Mechanism of Action

Approval under the Animal Rule requires a reasonable understanding of the investigational drug's mechanism of action with regard to its ability to prevent or substantially reduce the toxic effects of the challenge agent.[66] The sponsor should relate the mechanism of action of the drug in the proposed animal species to the presumed mechanism of action in the human. This information is critical to the selection of appropriate animal species in which to test the efficacy of the investigational drug and the interpretation of the results of those studies, in order for FDA to conclude that the drug's effect in the animal model is reasonably likely to be predictive of the drug's effect in humans.[67]

[66] See 21 CFR 314.610(a)(1) for drugs; 21 CFR 601.91(a)(1) for biological products.

[67] See 21 CFR 314.610(a) for drugs; 21 CFR 601.91(a) for biological products.

An understanding of the mechanism of action of the investigational drug may help in the identification of specific safety or efficacy issues, the interpretation of findings in the proposed animal studies, and the identification of additional studies that should be performed. This understanding also may lead to the identification of a relevant biomarker for potential use in selecting a dose and regimen in humans (see section V.B.2.b. for further discussion).

b. Drug Class

Information that is available about other drugs that are members of the same therapeutic class or pharmacologic class as the investigational drug can be used to help identify potential animal models. This information also may help anticipate safety and efficacy issues in the proposed animal model and in the projected human use.

c. Dosage Form and Route of Administration

The suitability of the dosage form and route of administration with regard to the proposed indication should be considered in the development of the drug. For example, an oral dosage form may be preferred for post-exposure prophylaxis for large populations while an intravenous dosage form may be more appropriate for seriously ill patients.

To the extent practicable, drug administration in the animal and human studies should be comparable to the expected clinical use of the investigational drug (e.g., dosage form, route of administration, to-be-marketed formulation). Comparative bioavailability information may be necessary to bridge pharmacokinetics across studies, for example, when changes in formulation occur during development. If multiple dosage forms or routes of administration are being developed, sponsors should discuss with the review division the types of PK data that may be needed to support the approval of each.

2. Selection of an Effective Dose in Humans

The Animal Rule requires that PK and PD data or information (or other relevant data or information) for the investigational drug[68] be sufficient to permit the selection of a dose and regimen expected to be effective in humans.[69] The methods used for selecting an effective human dose may differ based on factors including, but not limited to, the target of the investigational drug, prior human experience in related indications, and the availability of a relevant biomarker. Several approaches to the selection of an effective dose for humans are described in section V.B.2.b.

[68] This section focuses on the investigational drug as the active moiety; however, active metabolites also should be considered for the purposes of dose selection. Issues pertaining to active metabolites are handled on a case-by-case basis and should be discussed with the review division.

[69] See 21 CFR 314.610(a)(4) for drugs and 21 CFR 601.91(a)(4) for biological products.

Agency concurrence on the animal model in which the efficacy of an investigational drug will be tested will be contingent, in part, on the ability to extrapolate from the animal data to select an effective dose and regimen in humans. Sponsors are encouraged to initiate discussions with FDA on the proposed rationale for human dose selection early in their drug development program. Protocols for animal PK, PD, and efficacy studies should include adequate plans for assessment of PK and PD data for purposes of defining drug exposure and response characteristics.

Issues related to dose selection for the adequate and well-controlled animal efficacy studies for drugs and therapeutic biological products are discussed in section VI.B; for vaccines, see section VII.A.

a. PK and PD Information to Be Obtained in Animals and Humans

The absorption, distribution, metabolism, and excretion (ADME) of an investigational drug[70,71] should be characterized in animals and humans. In addition, protein binding characteristics and in vitro interaction potential (e.g., through inhibition, induction, or transporters) should be assessed. As in a traditional drug development paradigm, it is important to ascertain at an early stage of development whether a drug is eliminated primarily by excretion of the unchanged drug or by one or more routes of metabolism.[72] If elimination of the investigational drug is dependent in part on metabolism, the metabolites should be identified and the metabolizing route(s) should be understood.[73] This information will help identify potential interactions with medical products that are likely to be co-administered based on the clinical scenario and will help predict the consequences of metabolic differences among humans.

Studies should be conducted in healthy animals[74] and healthy human volunteers[75] to characterize the PK profile of the drug in each following the administration of a

[70] Biodistribution should be studied for certain products that are not biologically amenable to traditional ADME measures, such as cellular and gene therapies.

[71] Therapeutic biological products do not share the same ADME pathways as small molecules. The ADME characteristics of therapeutic biological products, including receptor-mediated clearance mechanisms leading to nonlinear pharmacokinetics, should be determined.

[72] Sponsors should discuss with the review division whether PK information in specific human subpopulations (e.g., renally and hepatically impaired) also should be obtained.

[73] FDA has issued a draft guidance on this topic. When the guidance on *Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations* is finalized, it will represent the Agency's current thinking on this topic.

[74] The healthy animals used in these studies should be representative of those used in the efficacy studies with regard to key animal characteristics, such as species/subspecies, country of origin, source, age, sex, and weight range.

[75] PK assessments in healthy volunteers may not be possible for some investigational drugs because of the nature of the drug, such as cellular therapies and gene therapies, or because of an unfavorable safety profile of the drug. In such cases, alternative plans should be discussed with the review division.

single dose and multiple doses (if applicable). The assays used for measuring drug concentration in the appropriate body fluids should be validated.[76] As in a traditional drug development program, clinical trials in healthy humans should evaluate safety and PK data over a range of doses. Based on nonclinical and human data, sponsors should discuss the appropriate upper limit for human dosing with the review division, and this agreed-upon upper limit should be used to support final human dose selection (see section V.B.2.b for further discussion). The drug exposures associated with efficacy in the adequate and well-controlled animal efficacy studies should be determined. PK information from affected animals[77,78] should be compared to PK information from healthy animals to determine whether the challenge agent-induced disease or condition affects the pharmacokinetics of the investigational drug.

The relationships between PK exposure parameters (e.g., area under the plasma concentration-time curve (AUC), peak plasma concentration (Cmax), trough plasma concentration (Cmin), and steady state plasma concentration (Css)) and PD parameters (e.g., efficacy endpoints and potential biomarkers) in the animal models should be determined over a range of at least three doses and the shape of the exposure-response (E/R) curves established in dose range-finding studies. To the extent practicable, protocols for the adequate and well-controlled animal efficacy studies should include plans for PK and PD assessments to enable quantitative E/R analyses.

When a biomarker is used as the basis for human dose selection, the assay method and performance characteristics for that biomarker should be described for the animal species and humans.

b. PK/PD Considerations for Human Dose Selection

PK/PD information can be informative in a number of ways. One approach to the selection of an effective dose for humans takes into account whether the effect of the investigational drug is mediated through its action on the etiologic or challenge agent, rather than on the host (e.g., antimicrobial drugs that target microbial pathogens or investigational drugs intended to bind or detoxify substances such as cyanide or neurotoxins). In such circumstances, it may be possible to use data from in vitro studies to estimate the target concentration or

[76] See FDA's guidance for industry *Bioanalytical Method Validation.* In the *Federal Register* of September 13, 2013 (78 FR 56718), FDA published a notice announcing the availability of a draft guidance for industry *Bioanalytical Method Validation* (Revision 1). This revised draft guidance updates the guidance for industry *Bioanalytical Method Validation* and, when finalized, will represent the Agency's current thinking on the topic.

[77] *Affected animals* are defined as those with the challenge agent-induced disease or condition of interest using the animal models proposed for the adequate and well-controlled efficacy studies.

[78] If there are barriers to performing intensive PK sampling in affected animals, sparse sampling approaches can be used.

exposure of the investigational drug.[79] The PK/PD parameters that correlate with activity of the drug should be identified in animal models. This information can guide the selection of doses to be tested in adequate and well-controlled animal studies to evaluate efficacy. Similar PK/PD parameters should be identified for humans to support human dose selection.

For example, in the case of antimicrobial drugs, in vitro studies can be used to determine a PD characteristic such as susceptibility (e.g., minimum inhibitory concentration (MIC), which is a measure of drug-organism interaction). Nonclinical studies then can be used to identify potentially relevant PK/PD parameters (e.g., Cmax/MIC ratio, AUC/MIC ratio, the time the concentration remains above the MIC (T>MIC)) that may correlate with an effective response.

If the investigational drug has been used in humans for other relevant indications, previously established human PK/PD information may guide dose selection for the animal efficacy studies, which in turn may support selection of the human dose for the proposed indication. For example, existing human E/R data from an antibacterial drug shown to be effective in pneumonia may guide the dose selection for the animal efficacy studies intended to support an indication for the treatment of pneumonic plague. Efficacy of the guided dose (e.g., the humanized animal dose) should then be evaluated in the animal model. In some cases, animal studies may suggest that the human dose and regimen needed for the new indication are different from the human dose and regimen used for other indications.

Another approach for human dose selection may be through the identification and use of an appropriate biomarker. The biomarker should be shown to be related to the mechanism by which the drug prevents or substantially reduces the etiologic or challenge agent-induced disease or condition and to the desired clinical outcome (i.e., reduction in mortality or major morbidity). In addition, there should be an ability to determine drug doses for humans that would result in biomarker levels in the desired range based on the biomarker levels associated with efficacy in the adequate and well-controlled animal studies.

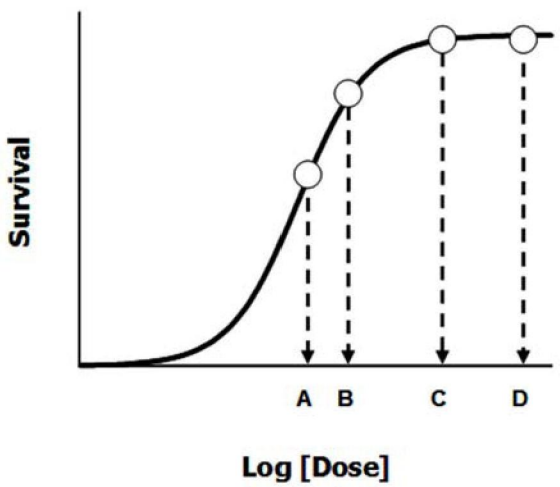
A common and challenging situation is one in which the relationship between the drug exposure and effectiveness is established in animals, but there is no evidence of a relevant link (e.g., biomarker, AUC/MIC) that can predict an effective drug exposure in humans. In this situation, it may be reasonable to assume that the E/R relationship[80] in humans will be similar to the E/R relationship in animals and use a conservative approach to human dose selection (discussed below), based on an

[79] The extent to which in vitro data may be relevant and useful varies; sponsors should discuss their supporting information with the review division.

[80] For the purpose of this guidance, the term e*xposure-response relationship* is used broadly to include dose-response relationship.

understanding of the E/R relationship in animals, the exposures associated with a fully effective dose in animals[81] (see Figure 1), and exposures associated with the agreed-upon upper limit for human dosing. This approach to human dose selection, based solely on comparing relevant exposure parameters (e.g., AUC, Cmax, Cmin, Css) between humans and animals, should be used only when there is no better alternative.

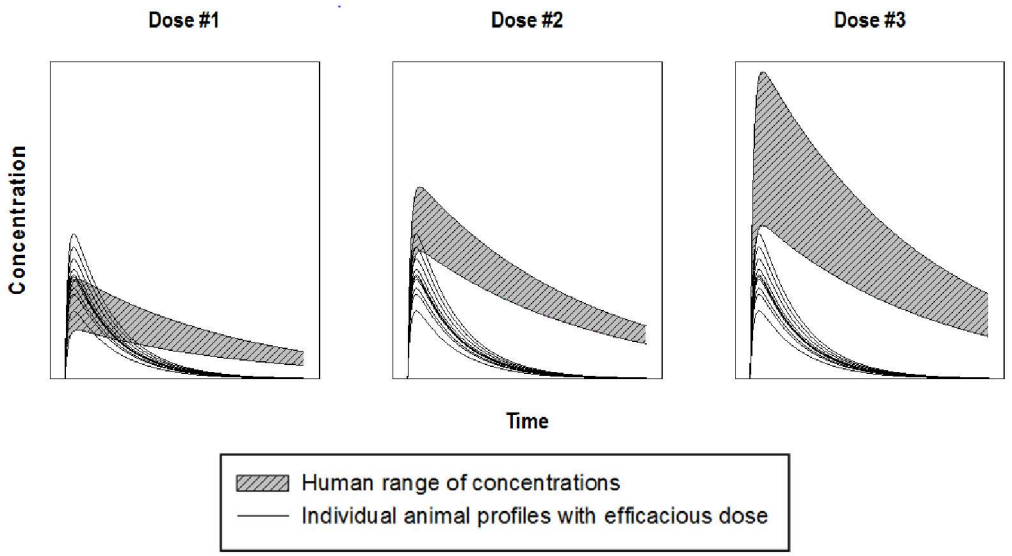
Figure 1 A Representative Dose-Response Curve for Survival Based on Four Doses of an Investigational Drug Studied in a Well-Characterized Animal Model



As depicted in Figure 1, survival is increased (compared to placebo) following administration of Doses A, B, C, and D of the investigational drug. The results of the testing of Dose D confirm that Dose C is a fully effective dose, because increasing the dose from C to D did not further increase survival. Although it may seem reasonable to use the exposures in animals resulting from the administration of Dose C to serve as the reference point for choosing the appropriate human exposure, when there is uncertainty as to whether the E/R relationship in humans is similar to the E/R relationship in animals, doses should be selected for humans that provide exposures that exceed those associated with the fully effective dose in animals, ideally by several-fold, if the drug's safety profile allows such dosing. To further minimize the possibility of sub-therapeutic exposures, human dose selection should also take into account the variability of exposure parameters in humans and healthy and affected animals so that any low values of exposure in humans will be greater than those associated with efficacy in animals.

[81] In most cases, the animal species requiring the highest drug exposure to demonstrate efficacy should be the basis for choosing the human dose.

Figure 2 Comparisons of Animal and Human PK Data to Support the Selection of an Effective Dose in Humans



In Figure 2, ranges of systemic drug concentration-versus-time profiles from human subjects following administration of three well-tolerated doses of an investigational drug are superimposed on the systemic concentration profiles from individual animals administered a fully effective dose. Based on a comparison of the animal and human PK data, Dose 3 represents an ideal situation with the full range of human exposures exceeding the exposures for each animal administered the fully effective dose, both for Cmax and overall exposure. If efficacy is not associated with the drug's Cmax, Dose 2 also represents an ideal situation. In the absence of scientific justification, Dose 1 is not acceptable because the full range of human exposures is not greater than the exposures associated with efficacy in animals. These situations are handled on a case-by-case basis and should be discussed with the review division.

Interspecies differences in ADME should be considered when determining the human dose. Differences in ADME between animals and humans may result in different systemic concentration-versus-time profiles among species,[82,83] that may necessitate adjustments in the dose or regimen tested in the adequate and well-controlled animal efficacy studies to achieve concentration-versus-time profiles that are similar to the profile observed in humans. Failure to account for

[82] Deziel, MR, et al., 2005, Effective Antimicrobial Regimens for Use in Humans for Therapy of *Bacillus anthracis* Infections and Postexposure Prophylaxis, *Antimicrob Agents Chemother*, 49(12):5099-5106.

[83] Kao, LM, et al., 2006, Pharmacokinetic Considerations and Efficacy of Levofloxacin in an Inhalational Anthrax (Postexposure) Rhesus Monkey Model, *Antimicrob Agents Chemother*, 50(11):3535-3542.

interspecies differences in pharmacokinetics may result in exposures in animals that are not achievable in humans, leading to the inability to select an effective dose in humans (see section VI.B for additional discussion). Differences in protein binding characteristics between animals and humans also should be considered, because only free drug, or the unbound fraction, is pharmacologically active. If the protein binding characteristics in the selected species differ from those in humans, comparison of free drug exposures will be relevant for dose selection.

Although not discussed further in this document, quantitative methods, such as PK modeling, PK/PD modeling, physiologically-based pharmacokinetic (PBPK) modeling, or population modeling, can be used to support the extrapolation of exposures in animals to doses in humans. The use of such methods should be discussed with the review division.

Sponsors should consider PK interactions in humans of the investigational drug with medical products likely to be used concomitantly in the clinical scenario. The sponsor, with knowledge of the ADME of the investigational drug, should discuss with FDA other medical products that are likely to be co-administered based on the clinical scenario and develop a plan to address the potential for human PK interactions using in vitro and in vivo assessments, if warranted. Potential combinations that may affect the pharmacokinetics of either drug should be considered for interaction studies. For example, if the investigational drug is metabolized via the cytochrome P450 system (CYP450), the safety or efficacy of the investigational drug can be compromised by the concomitant use of CYP450 inhibitors or inducers, and such drug-drug interactions should be evaluated. In the case of therapeutic biological products, the design and conduct of relevant drug-biologic interaction studies should be discussed with FDA with the overall goal of determining interactions with clinical impact.

When PD-based interactions (i.e., non-ADME based synergy or antagonism) with other drugs likely to be used in the anticipated clinical scenario have been identified, the sponsor should discuss with FDA the potential impact of these findings on the final human dose selection. For further discussion, see section VI.A, below.

VI. DESIGN CONSIDERATIONS FOR THE ADEQUATE AND WELL-CONTROLLED EFFICACY STUDIES IN ANIMALS

These animal efficacy studies substitute for efficacy trials in humans, and therefore, the assessment of efficacy in animals should follow best practices for adequate and well-controlled human efficacy studies, with endpoints that demonstrate an important clinical benefit, generally the enhancement of survival or prevention of major morbidity. When efficacy is demonstrated in a single study conducted in a sufficiently well-characterized animal model, FDA may require a

confirmatory animal efficacy study in that animal model.[84] The confirmatory study ideally should be conducted at a different laboratory; however, use of the same laboratory may be acceptable with justification. Supportive human efficacy data may negate the need for a confirmatory study. Early discussions between the sponsor and FDA about key study design elements and study conduct are highly recommended. Examples of key elements for discussion include the endpoints, proposed statistical analysis plan, and data quality and integrity plan (see the discussion on data quality and integrity in section IV.B). Agreement on these issues should be reached before the initiation of studies.

A. General Principles

Studies should be designed to mimic the ultimate clinical use of the investigational drug and to achieve meaningful outcomes similar to the effectiveness desired in humans. The animal studies should not use surrogate endpoints[85] as the sole evidence of efficacy. It is unlikely that surrogate endpoints will be persuasive to FDA because the Animal Rule requires that the animal study endpoint (generally, the enhancement of survival or prevention of major morbidity) be clearly related to the clinical benefit.[86] Analyses of secondary endpoints may contribute to an understanding of the disease or condition and a characterization of the treatment effect.

With rare exceptions, the adequate and well-controlled animal efficacy studies should evaluate the E/R relationship of the investigational drug, unless earlier studies have established the effective dose. For further discussion of dose selection in the animals, see section VI.B. The study duration is determined by the endpoint selected for the proposed indication. The study duration should incorporate adequate follow-up time to observe for recurrence of disease or condition after stopping drug administration. The route of administration of the investigational drug in animals should be the same as the route in humans, unless adequate justification is provided. Different dosing regimens in animals and humans may be needed to provide comparable exposure to the drug.

Animals of both sexes should be included and the male/female composition of the groups justified. FDA recognizes that there are significant supply constraints on the use of adult animals of certain species. The sponsor should discuss the age and the immune status of the animals used in efficacy studies, as compared to the intended human population. Inclusion and exclusion criteria for the acceptance of the animals into the study should be appropriate and prespecified before initiating the studies.

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[84] As stated in the preamble to the final rule, " . . . the animal studies should be replicated or substantiated in each species as needed to ensure credible results . . . " (67 FR 37988 at 37991, May 31, 2002).

[85] In this context, the term *surrogate endpoint* refers to a surrogate endpoint for efficacy, i.e., a drug-induced change in a biomarker that is considered reasonably likely to predict clinical benefit of the drug and that may be used in human clinical trials for a development program under the accelerated approval regulations (see 21 CFR 314.500 through 314.560 for drugs and 21 CFR 601.40 through 601.46 for biological products). Surrogate endpoints for efficacy are conceptually distinct from *humane endpoints*. Prospectively defined, objective euthanasia criteria that are necessary to address animal welfare are based on the selected humane endpoints (see Appendix A).

[86] See 21 CFR 314.610(a)(3) for drugs; 21 CFR 601.91(a)(3) for biological products.

The time course of observation should be optimized to assess the true treatment effect and to detect possible adverse effects. Animals should be monitored frequently; the frequency of observation may vary over the course of the study depending on the actual mechanism of disease or injury. In these studies that use mortality or major morbidity as an endpoint, observation frequency should be sufficient to ensure animal welfare and to minimize the potential loss or compromise of data.

Prospectively designed statistical analysis plans should be developed, incorporating the appropriate levels of statistical significance, including descriptions of the randomization procedures and methods to address missing data and, if applicable, outlying data. Protection against bias is critical in the adequate and well-controlled animal efficacy studies, just as it is in human trials.[87] Studies should be randomized. Variable block randomization is one approach to minimize bias in these animal studies that are frequently small in size. Euthanasia criteria should be prospectively specified and sponsors should provide a discussion of the potential effects of the criteria on the interpretation of results. Studies should be blinded. All personnel (e.g., investigators, veterinarians, animal caretakers, technicians) involved in making decisions regarding animal care interventions and/or euthanasia should be blinded. All personnel responsible for the collection, assessment, or interpretation of data obtained in the study (including but not limited to animals' clinical signs; vital signs; laboratory tests; procedures; imaging studies; and necropsy, gross pathology, and histopathology data) should also be blinded. Any situation in which study personnel may become aware of treatment assignments should be discussed with FDA in advance because of the potential for major effects on study interpretability.

For almost any situation in which the Animal Rule is used, there will be no basis for relying on a non-inferiority study to support effectiveness, and placebo-controlled animal studies should be used to demonstrate effectiveness. Data obtained in the placebo-control group of the efficacy study should be compared with the data obtained in the natural history or model characterization studies to substantiate the animal model. For example, if animals in the placebo-control group do not exhibit morbidity or mortality similar to that seen in the natural history studies, this may reflect a problem with preparation of the challenge agent that limits the ability to interpret outcomes in the active treatment arm(s) of the study.

If a drug has already been approved for the same indication and approval was based on the same animal species in which the investigational drug is being evaluated, the use of the approved drug in an active comparator arm, in addition to the investigational drug and placebo arms, is encouraged and should be discussed with the review division. The inclusion of the active comparator can be used to test for assay sensitivity (i.e., the ability of the study to differentiate an effective drug from an ineffective drug).

Investigational drugs should be evaluated within the context that reflects anticipated clinical use. The need for supportive care should be discussed with the review division early in drug

[87] See 21 CFR 314.126(b)(5) for drugs and 21 CFR 601.25(d)(2) for biological products.

development (see Appendix B).[88] When supportive care is used, the study should show that the investigational drug with supportive care is superior to placebo with supportive care. When incorporated into a study, supportive care should be administered either to all animals on a set schedule or to individual animals according to prospectively defined triggers, based on preliminary studies or available literature. When supportive care will be administered to individual animals based on prospectively defined triggers, the statistical plan should take into account the potential impact on the efficacy endpoint of differing supportive care among animals. The potential effects of the supportive care on the animal and on the pharmacokinetics and/or pharmacodynamics of the investigational drug should be considered in the design and interpretation of the study.

In addition, the sponsor, in consultation with FDA, should consider other drugs that are likely to be used in combination with the investigational drug in the clinical scenario and evaluate whether the activity of either the investigational drug or the concomitant medication, when used in combination, is affected by PD-based interactions (i.e., non-ADME based synergy or antagonism) and develop a plan to address the potential for such interactions. For example, it should be known whether the use of an anthrax antitoxin monoclonal antibody will have an effect on the activity of the antimicrobial drugs used for the treatment of disseminated anthrax, or whether the use of a drug that prevents replication of the target organism, resulting in a diminished immune response, may decrease the efficacy of a vaccine against that organism.

A checklist of elements of an adequate and well-controlled animal efficacy study protocol is provided in section X. In general, FDA should have the opportunity to review information on the proposed clinical indication, animal model, and method to be used to translate the effective exposures in animals to a dose and regimen in humans prior to detailed discussions regarding the design of a specific adequate and well-controlled efficacy study in animals. The design of an animal efficacy study should incorporate the principles discussed in sections IV and V. Protocols for these studies can be submitted with a request for review under the SPA provisions (see section III.A).

B. Dose Selection in Animals

The selection of the doses of the investigational drug[89,90] to be studied in the adequate and well-controlled animal efficacy studies should be based on an understanding of the E/R relationship in the proposed animal model. Dose range-finding studies should include at least three adequately spaced doses to help define the shape of the E/R curve, including establishing a fully effective dose (see Figure 1 in section V.B.2.b), that is, a dose that produces the largest effect the investigational drug can have. To identify a fully effective dose, it is almost always necessary to

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[88] When it is anticipated that supportive care will be used in the adequate and well-controlled animal efficacy studies, the assessment of similar supportive care in model development, including the natural history studies used to define the model, should be discussed with the review division.

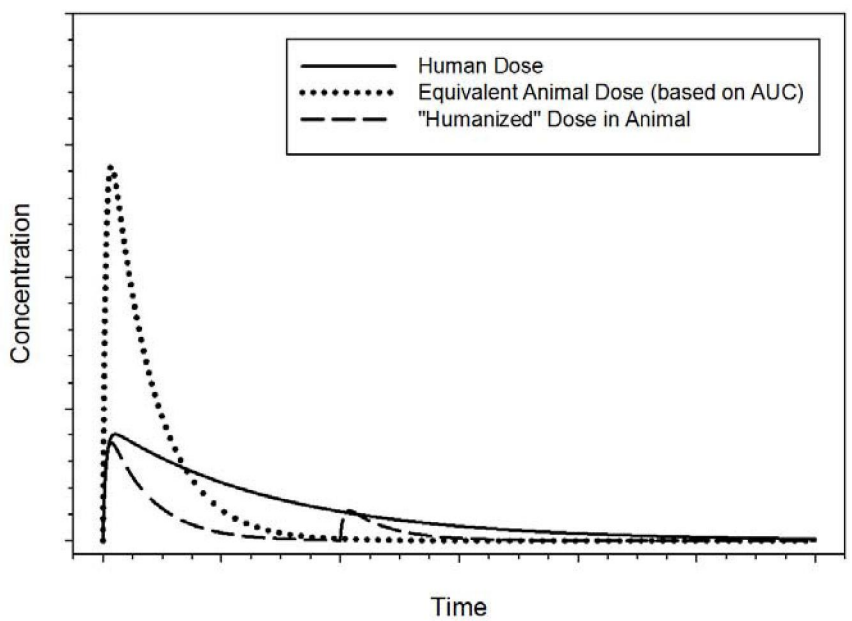
[89] This discussion assumes that the investigational drug is the active moiety. Issues related to active metabolites are handled on a case-by-case basis and should be discussed with the review division.

[90] For information on preventive vaccine dose selection, see section VII.A.

have studied a higher dose and shown no added benefit. For example, in Figure 1, the survival demonstrated with Dose D confirms that Dose C is a fully effective dose. At least one of the doses evaluated in the adequate and well-controlled efficacy studies should be a fully effective dose.

Prior to selecting doses for the efficacy studies, sponsors should understand the differences in ADME between humans and the selected animal species. Differences in ADME between animals and humans may result in different systemic concentration-versus-time profiles between species.[91,92] Failure to account for PK differences among species may result in exposures in animals that are not achievable in humans, leading to the inability to select an effective dose in humans. Some differences in systemic concentration-versus-time profiles between animals and humans may necessitate adjustments of dose regimens studied in animal efficacy studies to achieve concentration-versus-time profiles that are similar to the profile observed in humans. This is known as "humanization" of dose regimens and it is illustrated in Figure 3.

Figure 3 An Example of a "Humanized" Dose and Regimen for Evaluation in an Animal Model of Disease[93]



In this example, the shapes of the animal and human exposure profiles following once daily dosing are not comparable because the half-life of the drug in animals is much shorter than in

[91] Deziel, MR, et al., 2005, Effective Antimicrobial Regimens for Use in Humans for Therapy of *Bacillus anthracis* Infections and Postexposure Prophylaxis, *Antimicrob Agents Chemother*, 49(12):5099-5106.

[92] Kao, LM, et al., 2006, Pharmacokinetic Considerations and Efficacy of Levofloxacin in an Inhalational Anthrax (Postexposure) Rhesus Monkey Model, *Antimicrob Agents Chemother*, 50(11):3535-3542.

[93] Adapted from Bergman, KL, 2009, The Animal Rule and Emerging Infections: The Role of Clinical Pharmacology in Determining an Effective Dose, *Clin Pharmacol Ther*, 86(3):328-331.

humans. The dose regimen in animals is manipulated to achieve an exposure profile that is more similar in shape to that of humans. Adjusting the dose regimen used in animal studies based on differences in pharmacokinetics allows an improved comparison of exposures between animals and humans and, thus, provides greater confidence in selecting an effective dose in humans.

VII. CONSIDERATIONS FOR PREVENTIVE VACCINES AND FOR CELLULAR AND GENE THERAPIES

Although the overall principles of this guidance are applicable to vaccines[94] and to cellular and gene therapy products, additional considerations in the design of the animal efficacy studies exist because of the biological nature of these products. This section describes general considerations for study design and selection of relevant animal species for the adequate and well-controlled animal efficacy studies specific to vaccines and to cellular and gene therapy products. Before conducting an adequate and well-controlled animal efficacy study, FDA recommends that a sponsor request a meeting to discuss the details of the animal model(s) and study design, including the rationale and methods that will be used to extrapolate from a dose level(s) that shows substantial benefit in the animal studies to the final human dose and regimen.

A. Vaccines

FDA will rely on animal efficacy data for approval of vaccines using the Animal Rule only when the animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or the prevention of major morbidity. To estimate efficacy of vaccines in humans using the Animal Rule, the vaccine dose chosen for adequate and well-controlled animal efficacy studies should elicit an immune response in animals reflective of that in humans. Using pilot and proof-of-concept studies, a relationship should be established between the vaccine dose and the desired immune response, depending upon the study endpoint. The dose, route of immunization, and schedule may be different in the animal and human studies if the relevant immune response is similar, and adequate justification is made.

Sponsors should develop an approach for bridging animal responses to humans by careful selection of appropriate immune markers. Sponsors should accumulate as much immune response data as possible in their animal model(s), sufficient to characterize the immune response that is associated with the desired outcome of disease prevention. Such data can then be used to establish the vaccine dose and immunization schedule in humans needed to induce analogous immune responses. Sponsors should discuss with FDA their choice of an immune marker, which will depend upon the product and the animal model selected for these studies.

A single immune marker in an animal model may not reflect the spectrum of protective immune responses generated by humans. For example, for certain intracellular pathogens, animal models should be selected that demonstrate the induction of a protective antibody response as well as novel cellular immune response markers similar to humans. The choice of animal species should

[94] Cancer vaccines and therapeutic vaccines for non-infectious diseases are outside the scope of this guidance.

be made based on consultation with experts, review of the literature, discussions at scientific workshops and meetings, and discussions with FDA.

The challenge agent used in animal studies with vaccine products should be relevant to the human disease. When the etiologic agent's host range prevents the development of an acceptable animal model, studies should be conducted in animal models with closely related challenge strains, assuming that cross strain immune markers, such as cross reacting neutralizing antibodies, allow bridging to the human immune response. Ideally, the animal model(s) should show pathophysiology, progression of disease, symptoms, and host immune response similar to that observed in humans. Achieving this may call for optimization of the animal models in pilot and proof-of-concept studies using variable doses of the challenge agent to allow evaluation of the product's effectiveness and interpretation of the study endpoints in the adequate and well-controlled animal efficacy study(ies). Ideally, the route of exposure should reflect the anticipated route of human exposure (especially if the route of exposure significantly affects the pathophysiology, onset, and progression of disease). However, when the natural route of exposure is not known or cannot be replicated in a model, animal studies to demonstrate protective immune responses using other routes of exposure should be considered and discussed with FDA. Appropriate animal efficacy studies should be designed to provide information about the duration of protection afforded by the vaccine.

Sponsors should seek and carefully consider guidance from public health officials and experts concerning the intended use of the vaccine product. Either pre- or post-exposure prophylaxis clinical indications, or both, may be desired depending upon public health needs. Important immunization parameters, including the optimal dose, the schedule, and the desired time and duration of protection, may differ depending upon the indication. Studies supporting post-exposure use may be more technically challenging to design, depending upon the animal model. Vaccines used in a post-exposure scenario are expected to be given as soon as exposure is recognized. Therefore, post-exposure studies in animals should be designed to administer the vaccine at a time point considered relevant to exposure in humans and induce an immune response that can be extrapolated to humans and suggest clinical benefit. Data derived from pre-exposure prophylaxis studies could support the design of post-exposure animal studies, especially with regard to the kinetics and peak of the immune response. Sponsors should evaluate the possible concomitant use and resulting influence of therapeutic drugs and antimicrobial drugs on effectiveness of the product when designing studies of vaccines intended for use in post-exposure scenarios.

B. Cellular and Gene Therapies

1. Cellular Therapy Products

The selection of relevant animal species for evaluation of a cellular therapy product should include consideration of the host animal's response to the product, including inflammatory responses, innate and acquired immune responses, and interactions of the

cells with the host (direct and indirect biological responsiveness).[95] In addition, in vivo cell fate following delivery using the clinical route of administration should be characterized in each species. Cell fate includes cell distribution to target and non-target sites, survival/engraftment, differentiation and integration, phenotype, and proliferation. Administration of the cellular therapy product to healthy animals will not likely result in data representative of cell fate in humans. For example, in GI-ARS, cell turnover and mitotic rate will affect cell fate; thus, the response of the crypt cells to the cellular therapy pre- and post-radiation exposure will not be the same. In addition, if the cellular therapy product is delivered in combination with a matrix and/or scaffold or in an immunoisolation device, the biodegradation profile of these constructs should also be characterized.

If the cell fate, cell function, and/or host response to the cells in the animal species differs greatly from what is known or predicted in humans, administration of a well-characterized analogous cellular product in the animal studies may be considered.[96] The use of an analogous cellular product in an animal efficacy study is predicated on the ability to identify, harvest, and characterize (e.g., phenotyping and potency) a similar cell population in the animal species used for testing. Production of the analogous cellular product should meet the same standards as those applied to production of the final human cellular therapy product. Sponsors are encouraged to initiate discussions with FDA early in product development for guidance on the animal models and the potential use of an analogous cellular product prior to initiating the adequate and well-controlled efficacy studies.

2. Gene Therapy Products

The selection of relevant animal species for evaluation of a gene therapy product should include consideration of the host animal's response to the clinical vector, the expressed transgene, and/or the genetically modified cells.[97] Vector-specific issues include determining (1) the permissiveness and/or susceptibility of various animal species to infection and replication by the viral vector; (2) whether an immune or inflammatory response develops against the vector and if so, the effect of the response on the in vivo expression and persistence of the vector; (3) whether an immune response develops against vector positive cells; and (4) whether preexisting immunity to the vector exists in the animals.

Transgene-specific issues include determining (1) the pharmacological response of the species to the expressed transgene; (2) whether an immune or inflammatory response to

[95] For a more comprehensive discussion of the overall principles for the cellular and gene therapy products, refer to FDA's guidance for industry *Preclinical Assessment of Investigational Cellular and Gene Therapy Products.*

[96] As used in this guidance, *analogous cellular products* are defined as cellular products derived from the animal species used for testing that are analogs of the ultimate clinical product in phenotype and biologic activity.

[97] For a more comprehensive discussion of the overall principles for the cellular and gene therapy products, refer to FDA's guidance for industry *Preclinical Assessment of Investigational Cellular and Gene Therapy Products.*

the expressed transgene and/or protein develops; and (3) if an immune or inflammatory response does develop, the effect of the response on the in vivo expression levels, persistence, and functionality of the expressed transgene and/or protein in the animal species. If these transgene-specific factors significantly differ in the animal species from what is known or predicted in human cells and tissues, administration of the clinical vector modified to express an analogous transgene can be considered.[98] In such instances, product characterization comparison between the intended clinical construct and the animal homolog should be provided.

Issues related to genetically modified cells include (1) the sensitivity of the species to the biological actions of the modified cells and (2) the considerations conveyed in section VII.B.1.

VIII. HUMAN SAFETY INFORMATION

The Animal Rule neither replaces the need, nor establishes special requirements, for an adequate human safety database for drug development. The expectation is that drugs "will be evaluated for safety under preexisting requirements for establishing the safety of new drug and biological products."[99] FDA anticipates that the nonclinical and clinical safety development programs will proceed in a manner similar to that of drugs developed under traditional regulatory pathways. Some of the general principles include the following:

- Nonclinical toxicology, safety pharmacology, and PK studies (when relevant) should be conducted to provide adequate safety data to support the initiation of human trials.
- Risk-benefit assessment and ethical considerations must guide the design of human trials at each phase of development.[100] The regulatory and ethical complexities of establishing the necessary safety database should be discussed with the review division, preferably early in the drug development program.
- The size and composition of the human safety database should be consistent with the proposed use of the drug.
- The adverse event grading scale should be appropriate for the population to be studied (e.g., healthy adult human volunteers[101]).

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[98] As used in this guidance, an *analogous transgene* is defined as a transgene derived from the animal species used for testing that is an analog of the human-derived transgene in the clinical vector.

[99] See 67 FR 37988 at 37989, May 31, 2002.

[100] See Protection of Human Subjects regulations at 21 CFR part 50 and Institutional Review Boards regulations at 21 CFR part 56.

[101] The principles expressed in the following FDA guidance for industry may be useful: *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.*

- Safety signals identified from animal studies or human trials should be characterized, and if necessary, specific study design elements should be incorporated into the proposed nonclinical and/or clinical protocols to prevent or mitigate toxicity in future studies.

The evolving safety profile of the drug may necessitate changes in the clinical development program. When evaluating the available human and animal data at important steps during drug development, sponsors should determine whether the program remains on a suitable path to achieve an adequate human safety database and consult with FDA if necessary.

The size and composition of the human safety database necessary to support drug approval depend on issues such as the proposed indication, the drug's toxicity, and/or the extent of FDA's experience with a particular drug class. For a drug intended for the treatment of a specified life-threatening disease or condition, greater known risks or greater uncertainty about undefined risks may be acceptable when the drug offers a clear benefit for those patients. A database of at least 300 individuals will be needed for a 95% confidence interval to rule out a 1% rate of a specific adverse reaction (e.g., liver failure) if that specific adverse reaction did not occur in the population studied. In contrast, drugs intended for prophylaxis in large numbers of healthy persons with variable or unclear risk of disease or injury may require a safety database in the thousands to support a favorable risk-benefit assessment because little if any toxicity risk or undefined risks will be acceptable in this population. The numbers suggested above for the size of the safety database refer to individuals exposed to the drug using the proposed route of administration, dosage form, and formulation, and, at a minimum, the proposed dose, regimen, and duration.

For some drugs, there may be existing relevant human safety data. If the drug of interest is already approved, some of the existing safety data may be relevant to the proposed Animal Rule indication. For example, the safety information used to support the pneumonic and septicemic plague indications for Levaquin (levofloxacin) was obtained from the large safety database from its other approved indications. Similarly, if the drug of interest is in development for another indication, accrued safety data may be relevant for the proposed Animal Rule indication.

When an adequate safety database does not exist and human safety studies are needed, the risks should be carefully considered because the potential for benefit to individual human subjects participating in studies of drugs being developed under the Animal Rule is remote (i.e., the human subjects have no predicted prospect of exposure to the etiologic agent). Even a compelling need for a drug (e.g., an intentional release of a lethal or permanently disabling chemical, biological, radiologic, or nuclear substance) does not justify exposing study subjects to risks greater than those acceptable for other drug development programs. For drugs with only minor anticipated risks, studies in competent, appropriately consented adults are considered reasonable.[102] If concerns about safety and/or relevance limit the extent or usefulness of studies in healthy adult volunteers, sponsors should explore alternative approaches to contribute to the aggregate safety database and discuss them with FDA. In some circumstances, studies can be

[102] As stated in 21 CFR 56.111(a)(2), "Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may be expected to result."

conducted in existing patient populations for whom development of the drug may fill a need (even if the population is different from the intended target population).

Some safety concerns may not become apparent until the drug is used in the general population during an actual event. Examples include the potential for drug-drug interactions (e.g., a colony-stimulating factor and another drug that modifies the host immune system) and adverse interactions between the drug and a disease (preexisting or agent-induced). Such adverse interactions reinforce the critical need for postmarketing studies.[103]

Animal models used to demonstrate efficacy may not predict specific interactions of the agent-induced disease or condition and the investigational drug in humans. If adverse findings occur only when the investigational drug is tested in challenge agent-affected animals, further investigation may be warranted to determine the pathophysiological mechanism for the unexpected toxicity and its relevance to the risk assessment for the intended human population.

[103] Postmarketing studies to provide evaluation of safety and efficacy in the event an emergency arises and the product is used are required under the Animal Rule when such studies are feasible and ethical. A plan or approach for conducting such trials must be included with the NDA or BLA (for greater detail, see 21 CFR 314.610(b)(1) for drugs and 21 CFR 601.91(b)(1) for biological products).

IX. CHECKLIST OF ESSENTIAL ELEMENTS OF AN ANIMAL MODEL

The following checklist provides a list of data elements (and the corresponding sections within this guidance) for consideration when developing an animal model. The purpose of this checklist is to remind sponsors of the need to compare the data elements for the selected animal species to what is known about the human disease or condition in their submissions to FDA. Sponsors should note and explain any differences and indicate whether they expect these differences to have an impact on the interpretability of the data.

DATA ELEMENTS (Corresponding Sections Within the Guidance)	Animal(s)	Human
ELEMENTS RELATED TO THE ETIOLOGIC OR CHALLENGE AGENT-INDUCED DISEASE OR CONDITION		
CHARACTERISTICS OF THE ETIOLOGIC OR CHALLENGE AGENT		
• The Challenge Agent (V.A.1.a)		
• Pathophysiological Mechanisms of Toxicity or Virulence (V.A.1.b)		
• Route of Exposure (V.A.1.c)		
• Dose and Quantification of Exposure (V.A.1.d)		
HOST SUSCEPTIBILITY AND RESPONSE (V.A.2)		
NATURAL HISTORY OF THE DISEASE OR CONDITION - PATHOPHYSIOLOGICAL COMPARABILITY		
• Time to Onset (V.A.3.a)		
• Progression (V.A.3.b)		
• Manifestations (V.A.3.c)		
TRIGGER FOR INTERVENTION (V.A.4)		
ELEMENTS RELATED TO THE INVESTIGATIONAL DRUG AND THE SELECTION OF AN EFFECTIVE DOSE IN HUMANS		
THE INVESTIGATIONAL DRUG		
• Mechanism of Action (V.B.1.a)		
• Drug Class (V.B.1.b)		
• Dosage Form and Route of Administration (V.B.1.c)		
SELECTION OF AN EFFECTIVE DOSE IN HUMANS (‡)		
• PK and PD Information to Be Obtained in Animals and Humans (V.B.2.a)		
• PK/PD Considerations for Human Dose Selection (V.B.2.b)		
(‡) For information on vaccine dose selection, see section VII.A.		

X. CHECKLIST OF ELEMENTS OF AN ADEQUATE AND WELL-CONTROLLED ANIMAL EFFICACY STUDY PROTOCOL

This checklist is included to remind sponsors of the information that should be included in their adequate and well-controlled animal efficacy study protocols. For further information, refer to section VI.

PROTOCOL CONSIDERATIONS		
• Indication to Be Studied		
• Agency Concurrence on the Details of the Animal Model		
• Comparability of the Study Design to the Clinical Scenario		
STUDY DESIGN ELEMENTS	**Described**	**Justified**
• Controls		
• Size of Study Groups and Male/Female Composition of Groups		
• Animal Characteristics (†) (e.g., species, age, weight, source of animals)		
• Inclusion and Exclusion Criteria for Acceptance Into Study		
• Dose, Route of Exposure, and Preparation of the Challenge Agent		
• Trigger for Intervention		
• Dose, Regimen, and Route of Administration of the Investigational Drug		
• Randomization		
• Blinding		
• Statistical Plan		
• Endpoints		
• Euthanasia Criteria		
• Observation Frequency and Schedule		
• Animal Care Interventions		
• Plan for Ensuring the Quality and Integrity of the Data		
(†) See section IV.D for further description.		

APPENDIX A: GENERAL PRINCIPLES FOR THE CARE AND USE OF ANIMALS IN BIOMEDICAL RESEARCH

Animal studies must comply with applicable laws and regulations as prescribed by the Animal Welfare Act[104] and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.[105] FDA endorses the principles of replacement, reduction, and refinement of the use of animals in biomedical research.[106]

The following statements summarize general principles for the care and use of animals in biomedical research based on the animal welfare references listed at the end of this Appendix:

1. All persons involved in the use of animals in biomedical research should be appropriately qualified for and experienced in conducting procedures on living animals.

2. The living conditions of animals should be appropriate for the species and contribute to their health and comfort.

3. Unless otherwise established, procedures that cause pain or distress in human beings should be considered to cause pain or distress in animals. For such procedures, the following practices should be observed, unless there is compelling scientific reason precluding such practices:

    a. Appropriate sedation, analgesia, or anesthesia should be used during and/or following procedures that may cause more than momentary or slight pain or distress.

    b. Humane endpoints that do not jeopardize the scientific objectives of the study should be established to prevent animals from suffering unrelieved pain or distress.[107] Humane endpoints are the earliest indicators of severe distress, severe pain, suffering or impending death observed in an experimental animal.[108] Predetermined humane endpoints are used to develop objective euthanasia

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[104] See 7 U.S.C. 2131 et seq.

[105] U.S. Department of Health and Human Services, National Institutes of Health, Office of Laboratory Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Revised 2015, available at http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on October 16, 2015.

[106] Russell, WMS and RL Burch, 1959, *The Principles of Humane Experimental Technique*, London: Methuen and Co. Ltd. [Reissued: 1992, Universities Federation for Animal Welfare, Herts, UK].

[107] Humane endpoints are conceptually distinct from surrogate endpoints for efficacy. Surrogate endpoints for efficacy, discussed in section VI.A, should not be used as the sole evidence of efficacy in the adequate and well-controlled animal efficacy studies.

[108] Organisation for Economic Co-operation and Development, 2000, Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation, ENV/JM/MONO(2000)7, OECD, Paris, France.

criteria. Research necessitating endpoints for which pain and distress are not alleviated needs to be justified to, and approved by, the Institutional Animal Care and Use Committee (IACUC).[109]

c. Animals experiencing severe or chronic pain or distress that cannot be relieved should be euthanized painlessly. The appropriate use of euthanasia criteria is beneficial to the animal because unnecessary terminal distress is eliminated or significantly reduced. Also, it benefits the research effort because experimental goals can be met more consistently. Data collected after the development of severe physiologic derangements may not be useful or may be misleading for some purposes. Also, tissues that might otherwise be lost can be collected for postmortem analysis. Prospectively defined criteria for euthanasia should be included in protocol development. The criteria should be predictive of imminent death or specific moribund conditions and should be defined in objective terms that are relevant to the specific experiment.

d. For studies in which major morbidity or mortality are expected, observation frequency should be increased around the expected time of major morbidity or death to prevent animals from experiencing unrelieved pain or distress and also to minimize the potential compromise or loss of data.

4. Adequate veterinary oversight and care provided by a qualified veterinarian, as defined by the Animal Welfare Act, and involvement of the IACUC must be in place to ensure humane care and use of animals.[110,111,112] During protocol development, the attending veterinarian, or the veterinarian he or she designates, and the IACUC should play an active role in providing advice on humane endpoints and adequate veterinary care necessary to ensure the humane needs of animals are met and are compatible with the scientific requirements of the study, as determined through discussion with the principal investigator.

Animal welfare references include:

- The Animal Welfare Act[113]
- Guide for the Care and Use of Laboratory Animals, 8th edition[114]

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[109] See 9 CFR 2.31(d)(1)(iv)(A).

[110] See 7 U.S.C. 2131 et seq.

[111] See Health Research Extension Act of 1985, Public Law 99-158.

[112] See 9 CFR 2.31 and 9 CFR 2.33.

[113] See 7 U.S.C. 2131 et seq.

[114] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011, *Guide for the Care and Use of Laboratory Animals*, 8th edition, Washington, DC: National Academies Press (US).

- Public Health Service Policy on Humane Care and Use of Laboratory Animals[115]
- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training[116]
- AVMA Guidelines for the Euthanasia of Animals, 2013 edition[117]
- Recognition and Alleviation of Pain in Laboratory Animals[118]

[115] U.S. Department of Health and Human Services, National Institutes of Health, Office of Laboratory Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Revised 2015, available at http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on October 16, 2015.

[116] See 50 FR 20864, May 20, 1985.

[117] American Veterinary Medical Association, *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*, 2013, available at https://www.avma.org/KB/Policies/Documents/euthanasia.pdf, accessed on October 16, 2015.

[118] National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals, 2009, *Recognition and Alleviation of Pain in Laboratory Animals*, Washington, DC: National Academies Press (US).

APPENDIX B: TYPES OF ANIMAL CARE INTERVENTIONS

As described in this guidance, animal care interventions incorporated into animal studies are divided into three categories based on the rationale for their use: (1) intervention as part of adequate veterinary care, (2) intervention as supportive care to mimic the human clinical scenario, and (3) intervention to permit the manifestation of the disease or condition for the purpose of model development. These categories of interventions are discussed here:

Intervention as part of adequate veterinary care: Animal studies must comply with applicable laws and regulations as prescribed by the Animal Welfare Act[119] and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.[120] In addition, all studies should comply with general principles for the care and use of animals in biomedical research (see Appendix A for details). Compliance with these laws and general principles ensures that adequate veterinary care is provided, such that animals experiencing more than momentary or slight pain or distress are provided relief through appropriate analgesia, treatment, or, when prospectively defined criteria are met, euthanasia. Exceptions to this standard are permitted only when scientifically justified and approved by the IACUC. The standards for adequate veterinary care also include treatment of unexpected events, such as injury or the development of an unrelated disease. An example of an intervention that is considered part of adequate veterinary care is the administration of analgesics in a study assessing the effects of an investigational drug on vesicant-induced effects on the skin.

Intervention as supportive care to mimic the human clinical scenario: Supportive care, as defined in this document, is needed only to mimic, to the extent possible, the human clinical scenario. In general, it is relevant only for efficacy studies designed to support treatment of the disease or condition and the natural history studies on which the animal model is based. Animal supportive care can range from minimal intervention (particularly in the case of small rodents) to comprehensive medical support; however, it is not necessarily equal to patient care in a human clinical setting and in many cases may be significantly less intensive. The ability to provide certain types of supportive care can be species dependent (e.g., the ability to provide blood transfusions in a nonhuman primate model versus a rodent model). When included in an animal efficacy study, supportive care ideally should reflect the intended conditions of use of the investigational drug. It also should reflect the intended types of medical intervention and the timing of the availability of medical intervention expected in the human clinical or incident setting. The anticipated supportive care should be adapted, as appropriate, from the standard of human clinical practice to the animal species used, such as modifying the doses, route of administration, or the specific medical products administered.

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[119] See 7 U.S.C. 2131 et seq.

[120] U.S. Department of Health and Human Services, National Institutes of Health, Office of Laboratory Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Revised 2015, available at http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on October 16, 2015.

When supportive care is administered to the animals as part of the design of the efficacy study, the study should show that the investigational drug with supportive care is superior to placebo with supportive care. When incorporated into a study, supportive care should be administered either to all animals on a set schedule or to individual animals according to prospectively defined triggers, based on preliminary studies or available literature. When supportive care will be administered to individual animals based on prospectively defined triggers, the statistical plan should take into account the potential impact on the efficacy endpoint of differing supportive care among animals. The potential effects of the supportive care on the animal and on the pharmacokinetics and/or pharmacodynamics of the investigational drug should be considered in the design and interpretation of the study.

Intervention to permit the manifestation of the disease or condition for the purpose of model development: To study certain diseases or conditions, interventions are needed to permit the manifestation of the disease or condition of interest. Interventions used in this way are essential parts of the model development. For example, to establish a model of GI-ARS, it is necessary to attenuate the potentially lethal effects of H-ARS that occur before, or concomitantly with, GI-ARS. The interventions used to attenuate the H-ARS (e.g., partial bone marrow shielding during irradiation or bone marrow transplantation) are considered to be components of model development.

APPENDIX C: GENERAL EXPECTATIONS FOR NATURAL HISTORY STUDIES

Natural history studies are studies in which animals are exposed to a challenge agent and monitored to gain an understanding of the development and progression of the resulting disease or condition, including parameters such as manifestations (e.g., signs, clinical and pathological features, laboratory parameters, extent of organ involvement, morbidity, and outcome), the time from exposure to manifestation onset, time course and order of manifestation progression, and severity. Ideally, natural history studies should be prospectively designed,[121] adequately controlled, well-documented, and statistically powered to demonstrate the anticipated morbidity or mortality. In addition, the studies should include a statistical analysis of potential treatment triggers or critical determinants of disease or condition such as signs, endpoints, or biomarkers. Challenge dose standardization should occur before, or as part of, the natural history study.

In general, natural history studies should include randomized concurrent controls (i.e., unchallenged control animals) to reduce experimental bias (e.g., age- and sex-matched controls, or controlling for the effect of vehicle on the respiratory tract of experimental animals in aerosol challenge models). Blinding should be used, to the extent possible, to reduce investigator bias. Observation times and/or frequencies should be specified in the study protocol and should be based on available information and/or preliminary studies. The frequency of observation should be adequate to characterize the course of disease or injury and to define the desired endpoints and treatment triggers. The frequency of observation may vary over the course of the study, depending on the actual mechanism of disease or injury. Observation frequency should be increased around the expected time of major morbidity or death to ensure animal welfare and to minimize the potential loss or compromise of data. Findings from the natural history studies should be substantiated through replication of the study or a demonstration of results consistent with other relevant studies. For example, the median survival at a relevant time point and time to the development of neutropenia following exposure to a specified dose of whole body radiation should be similar for irradiated rhesus macaques in the natural history studies and in the control groups for the associated efficacy studies.

The natural history studies should be adequate in design, conduct, and reporting. These studies, designated for drug development under the Animal Rule, will be subject to inspection and audit by FDA to verify the reliability of the data. The expectations for data quality and integrity for model-defining natural history studies submitted for qualification are discussed in section IV.B.

The general expectations with regard to the animals used in the investigation, study conduct, the study report, and the submission of the study report and data are discussed in section IV.

[121] When it is anticipated that supportive care will be used in the adequate and well-controlled animal efficacy studies, the assessment of similar supportive care in model development, including the natural history studies used to define the model, should be discussed with the review division (see section VI.A and Appendix B).

APPENDIX D: ACRONYMS AND ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
AMQP	Animal Model Qualification Program
AUC	Area under the plasma concentration-time curve
BARDA	Biomedical Advanced Research and Development Authority
BLA	Biologics license application
BPCA	Best Pharmaceuticals for Children Act
BSL	Biosafety level
CBER	Center for Biologics Evaluation and Research
CBRN	Chemical, biological, radiological, and nuclear
CDC	Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
Cmax	Maximum (peak) plasma concentration
Cmin	Minimum (trough) plasma concentration
Css	Steady-state plasma concentration
COU	Context of use
eCTD	Electronic common technical document
CYP450	Cytochrome P450
DDT	Drug development tools
E/R	Exposure-response
EUA	Emergency use authorization
FDA	U.S. Food and Drug Administration
FDASIA	Food and Drug Administration Safety and Innovation Act
FD&C Act	Federal Food, Drug, and Cosmetic Act
FR	Federal Register
GI-ARS	Gastrointestinal subsyndrome of acute radiation syndrome
GLP	Good laboratory practice
H-ARS	Hematopoietic subsyndrome of acute radiation syndrome
HHS	Department of Health and Human Services
IACUC	Institutional Animal Care and Use Committee
IND	Investigational new drug application

LD_{50}	Lethal dose sufficient to kill 50% of those exposed to the agent
MCMi	Medical Countermeasures Initiative
MIC	Minimum inhibitory concentration
NDA	New drug application
PBPK	Physiologically-based pharmacokinetic
PD	Pharmacodynamic
PK	Pharmacokinetic
PREA	Pediatric Research Equity Act of 2003
SPA	Special protocol assessment
SNS	Strategic National Stockpile
USC	United States Code