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General Considerations for Animal Studies for Medical Devices

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

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When final, this guidance will supersede "Guidance for Industry and FDA Staff: General Considerations for Animal Studies for Cardiovascular Devices" issued July 29, 2010.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Office of Compliance

Preface

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Table of Contents

General Considerations for Animal Studies for Medical Devices

Draft Guidance for Industry and Food and Drug Administration Staff

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish 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 staff responsible for this guidance as listed on the title page.

I. Introduction

FDA has developed this guidance document to assist industry in designing evaluation strategies for, and reporting the results of, animal studies for medical devices. The animal studies utilized for the assessment of these devices typically provide initial evidence of device safety, their potential performance when used in a living system, and the biologic response that a living system may mount towards the device. This guidance provides recommendations for members of industry who perform, and FDA staff who review evaluations of, animal studies for medical devices. In this document, the terms "you" and "your" refer to members of industry, also known as "sponsors" or "applicants." The terms "we," "us," "our," and "Agency" refer to FDA.

The intent of this guidance is to provide a reference of best practices for the approach to and conduct of animal studies, and the presentation of animal study data intended to demonstrate that the device under study is sufficiently safe for early human experience [e.g., to support an investigational device exemption (IDE) application] or to demonstrate device safety in support of a marketing application, while incorporating modern animal care and use strategies. We recommend that you use this guidance to develop and present animal study protocols, methods, and reports that support the safety and performance[1] of medical devices. When considering the number of animals and the amount of data that can support the safety and performance of a medical device, FDA recommends balancing the ethical principles of reduction/replacement/refinement as well as regulatory least burdensome principles, with the goal of using the minimum number of animals necessary to generate valid scientific data to demonstrate reasonable safety and performance.

[1] While the handling/performance of a medical device may be demonstrated in an animal model, additional data in a human model may be necessary to assess outcomes demonstrating device effectiveness.

Although this document is not intended to address the regulations and policies of other agencies, or other laboratory animal guides, we note that there are other relevant regulations and policies involving animal care and use that are administered by other agencies, some of which are referenced in this guidance. [1-4] A summary of relevant federal regulations is provided in Appendix E, and additional resources on animal care and research are provided in Appendix F. Of note, FDA maintains a memorandum of understanding (MOU) with the U.S. Department of Agriculture (USDA) and the National Institutes of Health (NIH) that addresses common areas of regulatory interest concerning animal care and use.[5]

This draft guidance, when finalized, will supersede the July 2010 guidance entitled "Guidance for Industry and FDA Staff: General Considerations for Animal Studies for Cardiovascular Devices."

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

II. Scope

This guidance applies to medical devices intended for use in humans, as defined in section 201(h) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). The recommendations in this guidance apply to animal studies submitted in support of an IDE application, premarket approval (PMA) application, premarket notification (510(k)), humanitarian device exemption (HDE) application, or a request for *de novo* classification.

This guidance is intended specifically to apply to *in vivo* nonclinical laboratory studies as defined in 21 CFR 58.3(d). A list of common acronyms encountered in relation to these studies is provided in Appendix A.

III. Overview

FDA recommends that you consider the following general principles when developing animal study protocols for medical devices:

- For animal studies that are to be submitted to the Agency to support the safety of a medical device, Good Laboratory Practice (GLP) applies (21 CFR Part 58). If your animal study was not conducted in compliance with Part 58, your statement provided in your submission explaining the reasons why the study was not in compliance with GLP regulations should also describe in detail all deviations from the regulations. The statement should include information that will help FDA reconstruct the study, explain any confounding variables, and demonstrate that authentic and complete test data have been collected and reported.

- The animal model selected should be generally accepted for the study of the device type.[6 -12] There should be a reasonable amount of scientific evidence that the animal model has utility for the study of the device type. In some cases there may not be an established or accepted animal model for a specific device type. We recognize that the utility of animal testing may be limited in these situations, and it may be most appropriate to proceed with limited clinical evaluation in humans, if scientifically justified. In other cases, an alternative animal model may be used and appropriately justified.

- FDA's primary purpose in recommending an animal study is for the applicant to provide evidence of safety, including performance and handling. Note that in many cases, the performance of a particular device is intricately linked to its safety, such as for products that provide circulatory support.

- A secondary objective for conducting the animal study can be to evaluate the efficacy of the device or to demonstrate proof of principle.

- The *in vivo* setting generally provides an initial assessment of how the device interacts with biologic systems and also how the biologic system may affect the device, such as via device corrosion and structural deformities.

FDA is available to review your rationale for and design of an animal study as part of a Pre-Submission.[13] Additionally, it is important to consider the following points when designing your study: adequacy of controls, timing and route of intervention, and methods to minimize bias (e.g. blinding, randomization, use of controls, sample size based on expected magnitude of the biological response, reporting missing data, and clearly stated statistical considerations). If you are uncertain regarding elements of the animal study that are important to the Agency, please initiate contact with your respective review division for clarification.

You should observe the best practices of refinement, reduction, replacement, and current standards of humane veterinary care and use. This may also involve consideration of available validated alternatives.[14- 20]

Recommendations regarding specific elements of animal studies and other considerations are provided in the following sections.

IV. Study Planning and Protocol

FDA believes that an animal study that is carefully planned and executed is more likely to provide useful data in support of a device premarket submission. In this regard, the study should be planned by individual(s) with appropriate credentials and experience, and must be directed by a designated study director with appropriate credentials and experience in accordance with 21 CFR 58.33. The study director should be located in close proximity to the actual study location so that s/he can provide oversight for the technical conduct of the study. The study director is also responsible for the interpretation, analysis, documentation and reporting of the study results

(21 CFR 58.33). In some cases, additional investigators or contributing scientists may need to be designated for different aspects of the study, e.g., in-life portion and *ex vivo* imaging.

Because the primary purpose of the study is to evaluate safety and performance, we recommend you consider your risk analysis (i.e., the identified risks associated with your device through bench testing, and other information, such as scientific presentations, literature review, etc.) and design the study objectives to enable study of all identified risks of your device as well as any known risks of the device type.

The study must be guided by an *a priori* study protocol that is approved by the sponsor and signed and dated by the study director (21 CFR 58.120). The protocol must contain the elements outlined in 21 CFR 58.120 and should contain study instructions as dictated by the particular circumstances. Any changes or revisions to the final approved protocol, and the reason for the change, must be documented, dated and signed by the study director (21 CFR 58.120). The protocol and any revisions must be available for Agency review and are subject to inspection (21 CFR 58.15).

FDA recommends that an Institutional Animal Care and Use Committee (IACUC) review and approve all elements of the *a priori* protocol that address animal care and use prior to the initiation of the study and any major protocol amendment that affects animal care or use before the change is implemented (such review and approval may be required for some studies[1]). The IACUC will provide guidance as to the process and format for providing that information to the Committee.

The number of animals and experimental groupings should be designed after pilot and bench testing provide some idea of reliability and outcome. A thoughtful attempt at utilizing the least number of animals that will provide meaningful interpretation is paramount and includes such measures as attention to the appropriate experimental control, consideration of potential experimental confounders, and an idea of best observation intervals (See Appendix C).

V. Elements of the Animal Study

We recommend that your regulatory submissions include a discussion of each of the following key animal study features, in addition to the requirements outlined in 21 CFR 58.185:

- introduction, including a rationale for the selection of the particular animal model;
- the study assurances (e.g., USDA registration, AAALACi accreditation, NIH Office of Laboratory Animal Welfare [OLAW] Assurance Statement number);
- the purpose of each test protocol;
- the study schedule;
- any *ex vivo* tissue characterization; and

- any limitations imparted by the choice of the animal model and any amendments and deviations from the original test protocol.

Specific recommendations for how to optimize the development and reporting of some of these elements, as well as some of the elements required under 21 CFR 58.185, are provided below.

A. Rationale for Selecting Animal Models

FDA recommends that you provide your rationale for the selection of particular animal models for your animal study. A sample decision analysis flowchart for this determination is provided in Appendix B. The animal and its related environmental and physiologic attributes should provide a test system that offers a best attempt at simulating the clinical setting. The rationale for the conduct of an animal study should clearly state which of the elements of your risk analysis will be addressed and why the particular animal model was selected. If there are limitations to the animal model such that certain risks of the device are best addressed by bench or cadaver testing, these relationships should be described. Your rationale should also describe inherent challenges to the test system, such as:

- the similarities and differences between the test system and humans in the metabolism of drugs or the use of ancillary devices that represent the standard of care for the procedures utilized in device implantation;

- the dimensions of the device and delivery systems, as compared to the same characteristics of the device version intended for human use;

- the location of device insertion and the tracking pathway or, if surgically placed, the anatomic point of surgical entry and the surgical technique utilized in the animal versus the human; and

- size limitations that exist as barriers (exclusive of cost) to use of the most size-appropriate and anatomically appropriate model.

B. Study Assurances

Animal studies that are intended to support the safety of a medical device must comply with the GLP requirements detailed in 21 CFR Part 58. As part of these requirements, under 21 CFR 58.35, the Quality Assurance Unit (QAU) must be separate from and independent of the personnel engaged in the direction and conduct of the study. The final study report must contain the signed Quality Assurance Statement (QAS) (21 CFR 58.35 and 58.185), which should also be dated. The statement must also include dates of each inspection (21 CFR 58.35).

FDA recognizes that, for various reasons, use of a GLP facility may not be possible, such as when a highly specialized skill set of investigators is only available at a particular non-

GLP facility. In these situations, FDA recommends that you provide a complete rationale for the selection of the test site and that you follow the highest levels of oversight, record-keeping, and reporting. The rationale should include the differences from GLP and include an explanation as to why those GLP deviations do not affect the integrity of the data. If the reason for non-compliance with GLP is the lack of a QAU, FDA recommends that you employ an independent auditor so that impartial quality assurance is provided. For example, the quality assurance auditor should monitor the study conduct against the study protocol and facility standard operating procedures. The standard operating procedures should be similar in scope and detail as those typically used for GLP studies.

Finally, in situations where a study report and/or its appendices are lacking key data and information, if the study site has assurances such as USDA registration, AAALACi accreditation, and/or an approved Animal Welfare Assurance Statement with NIH, the scope and level of detail found in the test facilities' standard operating procedures may provide sufficient evidence to confirm the validity of statements in the final study report.

C. Study Objectives

FDA recommends that animal studies for medical devices be designed with the objective of studying the risks that are predicted from the design of the device, any known risks of the device type, and any new risks that may have emerged in prior investigations, such as bench testing or animal feasibility/pilot studies.

Recommendations for evaluating specific types of risks are provided below.

1. Performance and Handling

FDA recommends that your animal study protocol simulate the clinical setting as much as possible. You should identify all steps required to deliver, implant, or use the device, and develop acceptance criteria for each of the steps. FDA recommends that you apply a semi-objective rating scale (e.g., Likert scale) to each acceptance criterion. If the device is delivered or used with ancillary equipment, the acceptance criteria should include elements evaluating system compatibility. Rating criteria should encompass steps between the preparation of the device through device placement or use, and also withdrawal and redeployment, if appropriate. If the device is surgically placed, all steps from entry through the body wall through the final device handling should be described.

2. Device Safety

a. Physiological Response

Medical devices can cause mechanical or biologic stresses. FDA recommends that you identify key biologic response variables at regional sites, at locations adjacent to the implant site (if applicable), and along all paths to and from the point of implantation or use to develop active means of surveying the impact of your device on the body. FDA strongly recommends that you work with a

pathology expert such as a veterinarian boarded by the American College of Veterinary Pathology to develop the study protocol.

b. Unexpected Morbidity and Mortality

You should fully explain all observed instances of animal illness and death. The supporting rationale for any statements made regarding whether such events are or are not device-related should be thoroughly described. Retrospective testimonials or statements made by study directors, their designees, or their consultants that explain veterinary clinical outcomes should be supported by appropriate evidence, records, and reports. If the cause of death or illness could be indirectly attributed to the device, you should discuss the etiology of the condition. FDA recommends that you follow modern methods of animal health surveillance by having qualified veterinarians use problem-oriented veterinary medical records (POVMR)[21] for the purpose of detailing wellness or morbidity, including the development of key assessments for systemic effects of device use. These assessments include postoperative, interim, and terminal clinical pathology, including but not limited to: serum chemistry, hematology, and coagulation profiles with laboratory reference range values; imaging reports; and case report forms for specialized evaluations (e.g., electrophysiological, behavioral, and neurological assessments).

c. Downstream and Systemic Effects

FDA recommends that you evaluate whether or not the device can have effects remote from the site of placement or use. If you believe that your device has the potential for this type of risk, you should ensure that your study includes objectives to evaluate other tissue beds (such as downstream tissue for blood-contacting devices or other relevant end organ tissue) for evidence of potential systemic problem(s) that might be part of the device and delivery system. Should these findings occur, you should develop a plan for assessing the quantity of tissue affected and whether there are any resulting functional disturbances.

D. Study Schedule

FDA recommends that you develop a schedule of key interventions and time points for your study based on your knowledge of the known risks and predicted outcomes of use of the device. These timepoints typically include:

- full characterization, implantation, and intermittent examination of device performance and/or animal response;
- explantation of the device (if an implant);
- full analysis of any explanted tissue;

- preparation of the tissue; and

- preparation and sign-off of the final written reports.

We recommend that the QAU be aware of these key scheduling objectives so that interim study monitoring and inspections can be arranged. Because medical devices may involve some degree of invasiveness and predictable variability in animal survival, any anticipated change in the duration of study may necessitate adjustment of these parameters, depending on the interim data. For example, if adverse outcomes are detected at earlier time points than expected, you should consider enhancing the timetable for observation and device explantation so that useful terminal data are not lost. Also, we believe that the responsible use of animals optimizes the use of all animal tissue, and therefore recommend that complete gross and microscopic organ and tissue evaluations be performed on all animals and that tissue be freshly studied to avoid the potential for erroneous interpretation.

E. Test and Control Articles

Under 21 CFR 58.105 and 58.107, you are required to fully characterize and account for all test and control articles used in the study. Since sponsors may often develop several iterations of the test article prior to clinical study initiation, we recommend that pivotal animal studies utilize test articles representing the final clinical design. If the final design was not used, you should provide a rationale for why the final clinical design presents no new risks to the patient compared to the design studied in animals. FDA also recommends that test and control articles be packaged, sterilized, and shipped to the research site in the same manner as would clinical product. You should develop and follow a method for tracking the test and control devices from their manufacture or procurement to final use.

F. Accessory Devices and Equipment

Some test articles, such as vascular stents, are typically used in conjunction with specific or commercially-available accessory devices or components, such as guide catheters or guidewires. Such accessories are sometimes described as a part of the test system when their use is necessary to use the test article properly. We recommend that in such a case, you state if:

- any accessory devices used in the animal study are to be provided completely separate from the test article (i.e., commercially available), or if accessory devices will be marketed together with the test article (i.e., a kit); and

- whether the final labeling for the device will include instructions for accessory device selection or use.

G. Test System

The final study report must include a description of the test system (21 CFR 58.185). 21 CFR 58.3(i) defines *test system* as, "any animal, plant, microorganism, or subparts thereof to which the test or control article is administered or added for study." Additionally, FDA recommends that you provide a description of the following factors, as applicable, that may affect or influence the test system so that we can make a reasonable assessment of their contributions to the study outcome: the environment, including temperature, lighting, and physical structure; nutritional status; homeostatic controls, including electrolytes, blood glucose, maintenance of asepsis, and control of bleeding; ancillary diagnostic tools; and materials and methods used to define or describe the interaction between test or control article and the animal.

VI. Personnel

Each test report must contain a section that lists key study personnel (21 CFR 58.185(a)(10)). We believe that this information is relevant to regulatory review because 21 CFR 58.29(a) requires that study personnel are appropriately trained and experienced to properly carry out their duties. This regulation underscores the importance of the training and expertise of animal study personnel. FDA recommends that the animal study team include skilled clinical veterinary staff in order to detect and resolve adverse outcomes; make decisions about the necessity to intervene, intervene accordingly, or deviate from the protocol in the interest of humane care; preserve valuable tissue; and assist in the determination of device associations with any adverse finding. Animal models may frequently impart the need for unique surgical approaches, anatomical limitations, and important features of wound closure that best argue for trained veterinary surgical expertise as part of the research team.

FDA recommends that the animal study involve investigators with a combination of expertise, including human clinical, veterinary clinical, and veterinary pathologic fields. In keeping with the requirements in 21 CFR 58.29(b), you must maintain a current summary of the training and experience and job description of all personnel engaged in, or supervising the conduct of, animal studies. FDA recommends that any assessment of the competencies of key personnel be based on a rationale for why the individuals are suited for the type of studies being conducted.

FDA notes that appropriate training and experience of study personnel are also addressed in other relevant guides, and other agency regulations and policies.[1,1,4]

In addition to assembling a team of competent oversight personnel (including the study director, QAU, and attending veterinary and interventional staff), FDA recommends that you select the number of qualified personnel and their resources (including equipment, lateral and subordinate personnel, records and reports, and standard operating procedures) such that monitoring, treatments, and test sampling can be obtained at appropriate time points and to ensure that there is active surveillance at these periods for risks known or predicted in previous animal or bench testing, or possibly from previous experience with similar products. Finally, we recommend that you employ veterinary professionals with adequate training and experience to perform animal

welfare audits of facilities, personnel, and methodology for those business entities that you may wish to contract from, such as contract animal research or holding facilities.

VII. Facilities

A. Environment

We recommend that you consult published guidelines involving the housing and well-being of animal research models.[22-26] The referenced guidelines address recommendations for minimum housing, husbandry standards, social and environmental enrichment, and the development of standard operating procedures that address timely and adequate veterinary medical care. FDA believes that following these guidelines and allowing animals sufficient access to resources such as food and water receptacles, enrichment devices (toys), clean and species-typical resting surfaces, provisions for postural adjustments, and adequate play and exercise are important. Comfort and familiarity with handlers can reduce background stress, thus potentially minimizing experimental confounding factors that could adversely affect the interpretation of your study results.

In keeping with the standard of care, we recommend that the floors, walls, and ceilings of animal holding structures be non-porous in order to permit easy sanitization of surfaces. We recommend that there be adequate lighting and light controls to permit periods of normal daylight and opportunities for rest. We also recommend the utilization of facilities with appropriate environmental controls for temperature and humidity in order to prevent temperature stress and minimize respiratory infections.[4]

Additionally, we note that laboratory animal guides have been developed, and other agencies have established regulations and principles of humane animal care, including assurances to state, national, and international authorities that a state of animal wellness is maintained during research as a well-controlled test system.[1-3]

B. Animal Groupings

FDA regulation 21 CFR 58.43 requires testing facilities to have a sufficient number of animal rooms or areas, as needed, to assure proper separation of species or test systems. However, outside of the post-operative monitoring period, we recommend housing social animals in conspecific groups. FDA cautions that the environmental conditions not interfere with the assessment of the study and that all animals have access to adequate resources such as food, water, and toys in order to prevent bullying and territorial stress.

C. Primary and Secondary Enclosures

Because many Class III devices and implants associated with surgical procedures necessitate frequent observations during certain predicted sub-acute periods, FDA recommends that your facilities include access to small recovery rooms or enclosures that can provide intensive care treatments such as oxygen, swivel systems for intravenous

medications, remote ECG monitoring, and temperature and/or humidity adjustment. We also recommend that you consider whether your protocol should include periods of animal holding in high-level experimental facilities, with subsequent transport to more agricultural facilities following post-procedural stabilization.

D. Transport Systems

To minimize the stress animals can experience during transport, FDA recommends that you consider the use of transport cages with raised flooring, soft cushioning rest devices, carboys, hay nets, or other enrichment and food/water devices.[26,27] Transport vehicles should afford animals environmentally-controlled heating and air conditioning in order to further minimize shipping stress.[1,4] FDA notes that proper care in transport of animals is also addressed in other agency regulations.[1]

VIII. Study Methods and Conduct

FDA recommends that the methods and materials utilized for the assessment of medical devices in research animals be similar to those utilized in modern veterinary and human hospitals. Monitoring and intervention strategies should be based on the previous experience of key veterinary and scientific professionals. Once the failure modes and effects that can be addressed in an animal study have been identified, you should develop an animal study protocol that addresses each of the identified risks and that prescribes the frequency and type of monitoring, interventions, and outcome assessments.

A. Research Controls

Evaluation of device safety is often based on animal studies that provide valid scientific evidence (21 CFR 860.7(d)), and whether or not a facility has adequate standard operating procedures to ensure the quality and integrity of the data (21 CFR 58.81). FDA recommends that animal studies include adequate controls to minimize experimental variability and error. Such research controls include, but are not limited to, the minimization of anything given to or affecting the test animal in the course of an experiment that would impact the comparison between the test animals (i.e., animals receiving the test article) and control animals (i.e., animals receiving the control article). Variables that may impart change to the test animals may be devices other than the test article, or they may consist of background factors such as environmental factors, concomitant medications, or co-morbidities. You should minimize these confounding factors because they may hinder the ability of the investigator and FDA to clearly associate adverse or positive outcomes with the device and/or its effects.

With this consideration in mind, we recommend the use of personnel, consumable equipment, and practices that enable test article-associated outcomes to be clearly understood. A reference of key controls recommended for animal research studies is included in Appendix C.

B. Study Equipment

Given that a medical device animal study is typically sophisticated in its components, and in recognition of the shift from the use of sponsor-owned to contract study facilities, FDA recommends that study sponsors, their consultants, and the study director carefully assess the care, maintenance, and knowledge about the contract equipment used in the study. We encourage early and frequent interaction between personnel involved in the planning of the animal study and those who will actually perform the study. We believe this dialogue is especially important to ensure that the study facilities have the proper ancillary equipment, supplies, and resources for the study. For example, imaging equipment and personnel may need to be as advanced as those found in human interventional suites or operating rooms to properly emulate the clinical situation.

C. Animal Identification

You should include a table of information pertaining to animal identification, allocation to study sub-groups, type of procedure performed, and the fate or disposition of each animal. For example, if animals are purchased with a USDA identification number but then subsequently identified with an institutional identification number and then further described by a group number, this information should be clearly understood and equally well presented to FDA so that a chain of custody of any individual test or control animal is possible.

D. Animal Quarantine and Conditioning

FDA recommends that you implement standard operating procedures that permit for adequate periods of quarantine and acclimation, as well as a program of socialization.[4] Background levels of disease and psychological stress should be controlled as much as possible. Farm animals are particularly prone to intestinal parasites, which commonly present sub-clinically but can cause clinical syndromes under the stress of surgery and during recovery. To minimize this confounding factor, we recommend that you initiate early and frequent dialogue with the attending veterinarian about ways to detect and eliminate clinical and sub-clinical disease to ensure optimal animal wellness. We note that the laws and policies of other agencies, e.g., the Animal Welfare Act (7 U.S.C. §§ 2131-2159) and Public Health Service (PHS) "Policy on Humane Care and Use of Laboratory Animals," have resulted in important changes in the use of environmental and socialization protocols that are routinely implemented to control background stress.[1-2] We believe that following these laws and policies enhances the opportunity for and intensity of observations and can potentially result in other useful findings for the investigators.

E. Animal Allocation to Experimental Grouping

FDA recommends including a control group within the animal study design, or an explanation why a control group was not included. Additionally, when considering the number of animals needed to generate sufficient data that can support the safety and performance of a medical device, it is important to utilize sufficient animal numbers to obtain predictive outcomes. We believe that this determination can best be made after

bench testing is complete and the device iterations are finalized. We strongly recommend that you conduct definitive animal studies on the market ready device except as required to scale, if needed, to implant in the animal model. The number of animals in the study should be based on sound scientific justification with consideration for the difficulty of the model and whether one or more test article(s) and/or control article(s) can be reasonably studied in a single animal. For example, FDA believes that deployment and handling studies can often be performed multiple times in the same test subject, or incorporated into a chronic safety study. By contrast, studies involving high-risk implants such as prosthetic joints can involve a high degree of expertise and some expected morbidity, such that a relatively large number of animals may be appropriate in order to establish device safety. Based on our experience, typical animal studies in a higher species (e.g., sheep, goat, nonhuman primate) generally have 3-9 animals per group/time point. However, in all cases a scientific justification should be provided in the protocol for the numbers used. We encourage you to discuss proposed animal studies, including the number of animals to be involved, prior to implementation through the Pre-Submission process.[13]

F. Food, Water, and Basic Husbandry

FDA recommends that sponsors expressly communicate with subordinate and contract personnel the type and quantity of food that will be offered, and also to pre-specify that cage sizes, and the location and quantity of food receptacles should be ample in pen-housed situations. You should also consider following other research standards that more specifically prescribe housing limitations.[4]

We find weight loss challenging to interpret, making it difficult to attribute whether weight loss is or is not device related. As such, you should ensure that individuals monitor animals to document specifics regarding appetite, food and water intake, and micturition and bowel movements, particularly when animals are pen-housed. Bullying and resource coveting are commonly associated with weight loss due to inadvertent reduced caloric or fluid intake.

Animals (i.e., small ruminants) enrolled in chronic studies are often transferred to a more typical agricultural setting where animals are allowed to graze on open pasture and/or are fed hay as a component of their diet. The sponsor/test facility should ensure that the pasture is free of potentially poisonous plants, parasite ova and other potential contaminants, and that the condition (soil, grass) of the pasture meets the animal's nutritional requirements, including minerals. Some species may be sensitive to imbalances in organic metals in the soil (e.g., sheep are sensitive to copper and molybdenum imbalances) which may inadvertently lead to toxicities (copper toxicity in sheep). Growth-enhancing additives, such as monensin, are another common source of inadvertent toxicity due to errors in ration preparation or feeding a ration for one species to another. Feed and water used for the animals must be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed or water are not present at levels above those specified in the protocol (21 CFR 58.90(g)). The sponsor/test facility should be

cognizant of these potential problems and judicious in writing standard operating procedures that address periodic tests of water and feed for potential contaminants, the pasture soil and crop for nutritional balance, training employees on the importance of reading ration labels, feeding species-specific rations, etc. Local farm extension services provide invaluable assistance for this purpose.

G. Periods of Observation

FDA recommends that standards of veterinary care be followed.[1-3,7,28-31] For example, study animals should be monitored at a frequency and intensity that adequately assess for known risks posed by the device, and you should work with attending veterinary staff at the study facility to develop these monitoring parameters. We believe that such monitoring is appropriate not only for humane reasons, but also because well-monitored animals help us sort common spontaneously occurring conditions from conditions that might be attributed to the device. To best characterize the device effects on the animal, FDA recommends that the process be active and specific, rather than passive and general. Important attributes to consider for evaluation include, but are not limited to:

- respiratory rate, pattern, and depth;
- blood pressure;
- heart sounds and pulse character;
- mucus membrane color at rest and under exertion;
- attitude;
- mentation;
- gait; and
- presence or absence of abdominal, bladder, or bowel distension.

To best assist FDA's assessment of test article safety, we recommend that you follow current standards of record-keeping in veterinary medicine, such as the subjective/objective assessment and plan (SOAP) format. Additionally, these records should be readily available to all key support personnel in order to optimize data entry.

Specific recommendations for animal study monitoring are provided below.

1. Intraoperative Monitoring

Good surgical technique alone is not sufficient to ensure a successful outcome for complex procedures required for medical device implants. Intraoperative and

postoperative monitoring of heart rate, electrocardiogram, blood pressure, and blood gases are essential contributors to a positive outcome.

2. Acute Studies

If the study is acute and the device-associated trends are expected to be transient during the period of acute observation and harvest, we recommend that you track and record vital signs such as cardiac rhythm, respiratory rate, pulse oximetery, and blood pressure on operative records. This information should be correlated with the timing of insertion, implantation, deployment, or use of the device, contrast agent, or other device-associated materials, and noted on the anesthetic and/or operative records.

3. Chronic Studies

a. Post-Operative Period

FDA recommends that you follow the current standard of care for laboratory research animals by ensuring that investigators manage normal body temperature, minimize pain and infection, and provide adequate fluids and electrolytes.[4,30-34] You should capture physiological information similar in quality to that obtained in human care and recovery areas. In addition, you should control stress variables by establishing a standard assessment paradigm for the monitoring of pain and body temperature, and directing the administration of additional warmth and pain killers based on interim outcomes.

b. Interim Periods of Observation

During periods where animals have recovered from initial surgical procedures but are to be monitored for device-associated risks, FDA recommends that you monitor them at least twice daily at feeding times so that they may be observed when active. We also recommend that you consult your veterinarian and develop a weight monitoring plan. You should consider inclusion of body scoring as an adjunct to your periodic observations of the animal.[7,35-38]

If your study involves the collection of clinical chemistry data or more advanced diagnostics, we recommend that you develop standard operating procedures that prescribe, when needed, a method of chemical restraint that does not interfere with the device. In our experience, some animals, such as dogs and sheep, may be conditioned to be compliant for these activities, while swine rarely are.

c. Terminal Study Period

FDA recommends that the study protocol include details of the terminal study and include all methodology for the examination, collection and processing of tissue. This section of the protocol should include the following information:

- methods for end-period examination;
- (if applicable) a statement that in-life radiographic analysis and/or imaging will be completed; and
- methods for establishing end weight and/or body score.

d. Necropsy and Post-Mortem Evaluation

Adverse events may present clinically or subclinically; therefore, we recommend that you include a comprehensive systematic necropsy in your study, including tissue collection and preservation for possible processing for histopathology examination as the resulting information can help FDA to determine whether observed adverse events are device-related. FDA generally recommends that you describe the rationale and process for how sectioning of organs is performed and the training and experience of the prosector in order to assure an objective process in the sampling of gross tissue for microscopic evaluation. You should support any statements regarding whether any adverse outcomes are device-related with appropriate evidence from the necropsy or histopathology report and from in-life observations. In the event of an unscheduled death, you should be able to provide evidence that supports your statement regarding cause of death.

H. Post-Mortem Imaging and Assessment Methods

1. Explant Imaging (i.e., radiography, microCT)

Prior to preparing devices for histomorphometric analysis, you should consider whether an analysis of the structural integrity of the device would assist in the determination of device safety.

2. Scanning Electron Microscopy (SEM)

FDA recommends the use of Scanning Electron Microscopy to fully characterize the implant device surface after explant of the device from animals.

3. Histomorphometric Analysis

Because proper interpretation of acute and chronic biologic responses is critical to FDA's evaluation of safety, especially in the absence of clinical data, we recommend that you seek the expertise of board-certified veterinary or clinical pathologists when developing and executing methods for preparing tissues for histomorphometric analysis. We also recommend that you identify appropriate expertise to develop pre-specified objective methods for scoring and analyzing observations of injury and inflammation of all tissue. Specific assessments such as inflammation, vascularization, calcification, proteoglycan/collagen, and fibrin/thrombus, etc. should be considered in your evaluation.

FDA recommends that you report any non-standard tools and methods used to collect the tissues that contain the device as well as the methods of fixation, cutting, and staining. The reports should also include diagrams indicating the location of implants. The sectioning methods, including tissue and device orientation, should be detailed. When discussing the study results, you should include well-marked high resolution color images, each indicating the animal number, study group, tissue section, magnification, stain, and other important identifiers. Some sponsors find the use of pathology keys that further detail their grading system useful. Other important identifiers are experimental animal number and cohort as well as a scale on the photomicrograph.

4. Local and Downstream Tissue Assessment

FDA believes that most devices, including both implant and delivery system components, have the ability to embolize particulates or microthrombi from devices' structural elements or coatings, resulting in adverse observations such as pressure necrosis and inflammation in surrounding tissue or upstream/downstream tissue if the device is in contact with blood. The calvarium should be opened and the brain sectioned if there is a risk of upstream emboli. If your risk analysis identifies this potential risk for your device, we recommend that your pathologic study include systematic descriptive evaluation of upstream/downstream and surrounding tissue. If foreign bodies are observed, you should provide a discussion of the amount of surface area affected as well as the methods utilized to calculate this affected area.

IX. Records and Reports

A final report must be prepared, and any changes to the final study report must be documented as report amendments in accordance with 21 CFR 58.185. All raw data, documentation, protocols, final reports, and specimens (with certain exceptions) generated as a result of the animal study must be retained (21 CFR 58.190). FDA recommends that you prepare the records and reports for your animal studies such that we can most efficiently evaluate device safety and performance. You should consider whether the data are best suited for statistical analysis or better presented "raw." When raw data are requested by the Agency, you should include individual animal recordings and key study attributes as appendices to the final study report, and organize their format and content with the goals of explaining all study outcomes to minimize ambiguity. We recommend that the protocol also contain information about how the records will be organized and stored; who will make entries for each attribute; and when interim inspection of the records will be performed. We also recommend time and date stamping for study observations, as this helps to capture events accurately, which aids in the assessment of the inter-observational differences between study subjects. The final study report must include the information specified in 21 CFR 58.185, including a description of all circumstances that may have affected the quality or integrity of the data.

Under 21 CFR 58.190, you are required to retain all raw data, documentation, protocols, final reports, and specimens generated as a result of a non-clinical laboratory study for the durations specified under 21 CFR 58.195.

X. Preparation of Regulatory Submissions

When preparing regulatory submissions, including IDE, 510(k), HDE, and PMA submissions and *de novo* requests, we recommend that you include all relevant information collected as part of your animal studies. The summary of nonclinical studies in your submission should discuss the number of animal studies conducted, and include the following information for each of the studies:

- the rationale for the model selected;
- the similarity of the selected model compared to humans;
- the general animal study methodology you used;
- whether there were standard operating procedures in place and followed during the study; and
- how the quality assurance unit is independent and impartial with respect to the inspection of the data and the reporting of the results.

In addition, you should include your rationale for your transition from pilot, validation, or proof of concept animal studies to pivotal animal studies, or from one pivotal study to the next, as this information assists us in understanding how you comprehensively assessed device safety, and performance and handling across multiple studies. You should also describe any design changes to the device that were implemented after completion of all animal studies.

We recommend that you also provide a tabular representation of key parameters for each study, including the following information:

- the study groups;
- the number of animals in each group;
- identification of animals corresponding to study group allocation;
- study duration;
- the device design iteration used; and
- a summary of study outcomes.

A signed and dated copy of the final study report should be included in your submission with changes to the final study report documented as report amendments. You may also submit an overall report of the study. In addition, you should provide an attachment to each final study report for each animal study that includes study details, including signed and dated individual scientific reports (e.g., the study director, the clinical veterinarian, the pathologist, and the radiologist), accompanying test protocols, and raw data. These attachments should also identify key study personnel and facilities, describe the overall results of the study, and discuss how the results met the objectives of the study and demonstrated that the device is safe for human use. To aid with the presentation of this information, FDA recommends that your overall animal study summary identify and present the individual test reports in a tabular format, and provide the locations of relevant appendices and attachments to the final study report within the submission.

When compiling more than one study into a group of attachments to the final study report, FDA recommends that you do so in the order in which the studies were performed so that we can follow the device history and *in vivo* performance from the first to the last study, and evaluate the means by which you assessed device safety and performance and arrived at your final conclusions. A sample organizational template for relevant content of an animal study report for regulatory submissions is provided in Appendix D.

In addition to these considerations, we recommend that you review any available FDA guidance documents specific to your device type for more detailed animal study recommendations.

Appendix A: List of Common Acronyms Related to Animal Studies

AAALACi: Association for Assessment and Accreditation of Laboratory Animal Care, International

ACVIM: American College of Veterinary Internal Medicine

ACVECC: American College of Veterinary Emergency and Critical Care

ACLAM: American College of Laboratory Animal Medicine

APHIS: USDA Animal and Plant Health Inspection Service

CDRH: Center for Devices and Radiological Health

CFR: Code of Federal Regulations

FDA: United States Food and Drug Administration

GLP: Good Laboratory Practice (21 CFR Part 58)

IACUC: Institutional Animal Care and Use Committee

NHP: Nonhuman Primate

PHS: Public Health Service

QAS: Quality Assurance Statement

QAU: Quality Assurance Unit

SOAP: Subjective/Objective Assessment and Plan

USDA: United States Department of Agriculture

US: United States

Appendix B: Sample Decision Tree for Medical Device Animal Studies

1. Have you completed a risk analysis that considered all sources of relevant information, including your own knowledge of risks and failure modes that you believe exist with your device, risks commonly attributed to this general device type, and post-market information for similar marketed devices? Postmarket information can be obtained from the published literature and the CDRH Manufacturer and User Facility Device Experience (MAUDE) database (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfMAUDE/search.CFM).

    a. If yes, go to step 2.

    b. If no, we recommend that you complete the risk analysis and go to step 2.

2. Have all of the evaluable risks been tested on the benchtop, to the extent feasible, using the **final design** iteration (i.e., proposed market-ready device)?

    a. If yes, go to Step 3.

    b. If no and if feasible, we recommend completion of bench testing with the final device design before proceeding to step 3.

3. Did the risk analysis suggest that an animal study is necessary to assess potential safety problems?

    a. If yes, go to Step 4

    b. If no, consider submitting a Pre-Submission[13] and request FDA feedback.

4. Is there an established animal model for the type of device you are testing (i.e., one that has been described in the literature or used to support the clearance or approval of a similar device for the same indications for use)?

    a. If yes, go to Step 5.

    b. If no, have you assessed the anatomy and physiology (e.g., angiographic, radiographic, CT screening) of commonly utilized laboratory animal species (e.g., small hoofed stock, dogs, and nonhuman primates) for size and procedural approach features?

        i. If yes, and you can identify an animal model that would work, go to Step 5.

        ii. If yes, and you identify significant challenges that prohibit the use of a reasonable animal model for all or some of the animal studies recommended by the risk analysis, FDA recommends that you contact the Agency for a

discussion of these challenges and alternative approaches for collecting evidence to demonstrate satisfactory device safety and performance prior to clinical use via the presubmission process. Please note that FDA believes such situations to be unusual. As part of this discussion, you should include any available evidence that animal studies would not be feasible, propose alternative solutions, including any available simulations, cadaveric studies, and clinical information collected outside the United States. Please also note that FDA generally does not consider high cost as sufficient justification for not conducting animal studies.

iii. If no, FDA recommends that you consult an experienced laboratory animal veterinarian to determine the availability and utility of common laboratory species before proceeding to Step 5 or Step 4.b.ii.

5. Are there any particular features of the device that would result in study endpoints that differ from those previously used in studies for other devices of the same type for the same proposed indications, or are there new indications that suggest the use of different or additional evaluation time points or methods?

    a. If yes, you should identify the new endpoints, time points and methods, and proceed to Step 6.

    b. If no, FDA recommends that you use the endpoints, time points, and methods reported for similar devices, and proceed to Step 6.

6. Is there anything known about the device that would indicate high variability of animal responses, due to factors such as investigator training and familiarity with the device or inherent challenges in the placement or tolerance of the device?

    a. If you have investigated this issue and have determined that there is not a significant learning curve or predicted animal response variability, proceed to Step 7.

    b. If evidence exists from either *in vivo* or *in vitro* studies that a significant learning curve exists that would significantly increase animal response variability, FDA recommends conducting pilot or proof of concept animal studies to evaluate this issue prior to conducting pivotal animal studies and before proceeding to Step 7.

7. If, after consideration of all these issues, you would like FDA feedback on your proposed animal study strategy, FDA recommends that you submit a Pre-Submission[13] that includes a proposal for your pivotal animal studies. This proposal should detail all methods of assessment for identified risks that may be observed dynamically in life and with gross pathology and histopathology, and include any specific questions for which you would like FDA input.

Appendix C: Recommended Animal Study Research Controls to Consider

The requirements in 21 CFR Part 58 (e.g., adequate calibration and maintenance of experimental equipment in accordance with standard operating procedures, 21 CFR 58.63 and 58.81(b)(11), and proper identification of the test system, test article, control article, and all specimens collected from the test system to preclude error in data recording and storage, 21 CFR 58.105, 58.107(c), 58.120(a)(5), and 58.130(c)) are intended to ensure the quality and integrity of the data generated from the study. In addition to these requirements, we recommend that you consider implementing the following controls to help keep the study focused, with clear goals, and minimize problems that can interfere with a successful study.

- Whenever possible, use pilot studies to best aid in the selection of time points, animal numbers, and interventions that minimize confounding and optimize animal use. The number of animals to be used in the study should be stated with clear reasoning.

- In addition to defining the study objectives as required under 21 CFR 58.120(a), we recommend that you include *a priori* acceptance criteria for success that are based on clinically relevant risks (often identified in your Risk Assessment Plan). A plan for analysis of these criteria should be defined and should include, where appropriate, the statistical methods that are to be used with definitions of success and failure. If using a semi-quantitative rating scale, define the score required that constitutes "success/pass" and provide your scientific rationale.

- Ensure selection of normal healthy animals based on timely interpretation of laboratory work and veterinary medical examination prior to study enrollment.

- In addition to the requirement in 21 CFR 58.90(g) to analyze feed and water periodically for known contaminants, incorporate methods that permit nutritional adequacy for the species under study, such as:

  - regularly scheduled interim weight measurements

  - provision of adequate number of feeders in pen-housed animals

  - consultation with attending veterinary staff regarding provision of special feeds or special nutritional supplements during periods when you may expect finicky eating behavior, such as the peri-procedural time frame.

- Consider use of an acclimation period after the source animals arrive at the test facility, such as 7 to 10 days, prior to study enrollment.

- Incorporate appropriate baseline assessments of animal health and behavior prior to study enrollment, including timely veterinary interpretation (e.g., fecal examination for parasites,

hemogram, and blood chemistry accompanied by the laboratory reference values). Under 21 CFR 58.90(c), at the initiation of the study, animals must be free of any disease or condition that might interfere with the purpose or conduct of the study. Therefore, screen animals out or treat and verify medical readiness for study, thereby minimizing the inability to associate clinical pathology with the device vs. a pre-existing condition.

- Ensure proper aseptic surgical technique, and monitoring and intervention to control unintended infections.

- Incorporate practices and procedures, as appropriate, in addition to those required under 21 CFR Part 58, that ensure the animal facility staff are providing adequate sanitation and environmental controls to prevent unintended injury and infection.

- Incorporate practices to ensure that training in the planned experimental methods have exceeded the device learning curve, such that there is low to non-existent inter-procedural variability.

- Under 21 CFR 58.29(c), there must be a sufficient number of personnel for the timely and proper conduct of the study according to the protocol. Therefore, you should incorporate practices to ensure that there is adequate personnel and staffing to make certain that animals are appropriately monitored throughout the duration of study and at the appropriate intensity and duration that would reasonably detect the predicted failure modes as well as any common experimental outcomes.

- Ensure that the protocol includes appropriate monitoring and timely postoperative monitoring and intervention to detect, control, and report common physical and physiological outcomes such as vascular spasms, arrhythmias, respiratory difficulty, seizures, gait disturbances, cognitive dysfunction, pain, and distress.

- Incorporate practices to ensure that transportation and shipping stress is minimized when moving peri-procedural animals to remote holding sites.

- Incorporate practices and procedures that enable animals in group settings to consume adequate amounts of water and food and to minimize inter-species injury.

- Incorporate practices that encourage adequate and timely intervention to obtain complete necropsies (gross and histopathology) when animals die unexpectedly in order to establish whether the cause of death is or is not device-related.

- Incorporate practices that encourage proper handling, storage, and preparation of tissue for chemical analysis and histological processing.

- Consider steps to minimize bias or the perception of bias including, but not limited to:

    - Contributing Scientists with no financial conflicts,

- Blinding, and
- The utilization of more than one observer.

- Incorporate programs that will provide physiologic homeostasis, such as adequate thermoregulation, and electrolyte, blood glucose, and caloric balance.

- Incorporate a program to maximize animal wellness through the provision of species-specific social adequacy and environmental enrichment.

- Incorporate procedures that standardize the timely methods for the collection, handling, and shipment of tissue specimens.

Appendix D: Sample Organization of Animal Study Test Report [Including Raw Data (as defined by 21 CFR 58.3(k))] Components to Facilitate Review

The list below is intended as an example of the organization of a test report for recommended and required content. This sample does not include all of the requirements of 21 CFR 58.185. For more information on required content necessary to be in compliance with Part 58 reporting requirements, please see 21 CFR 58.120 and 58.185.

1. Report numbers (as applicable)
    a. Institutional Animal Care and Use Committee/Ethics Committee protocol number
    b. Study director protocol number(s)
    c. Test Facility protocol number(s), if applicable
2. Title of the report
3. Description of compliance with GLP regulations. If not in compliance, your statement provided in your submission explaining the reasons why the study was not in compliance with GLP regulations should also describe in detail all deviations from the regulations. The statement should include information that will help FDA reconstruct the study, explain any confounding variables, and demonstrate that authentic and complete test data have been collected and reported.
4. Contact information (e.g., mailing address, street address, city, state, country and zip code for each contact)
    a. Sponsor
    b. Sponsor representative
    c. Test facility name(s); provide additional information, if available:
        i. USDA registration (yes/no)
        ii. AALACi accredited (yes/no)
        iii. PHS Assurance (yes/no)
    d. Study director
    e. Quality Assurance director
5. Final report signature

a. Study director's signature

b. Quality Assurance Statement and signature

6. Copy of the protocol reviewed by the Institutional Animal Care and Use Committee (IACUC), and signed by the IACUC chairperson and attending veterinarian

7. Executive summary

    a. Overview of animal study

        i. Study Schedule

        ii. Objective of the study

        iii. Acceptance criteria

        iv. Rationale for selection or exclusion of animals, including supporting discussion and rationale if the proposed animal model could not be used

        v. Characterization of test and control articles

            a) Design iteration of device used

            b) Referenced serial or model numbers

        vi. Brief discussion of methods used, including insertion, approach, incision, monitoring, intervention, imaging, necropsy, and histology as appropriate

        vii. Brief overview of results

            a) Morbidity/mortality

                (i) Gross necropsy information

                (ii) *In situ* photography

                (iii) Descriptive findings

            b) Biologic response to the device to include such things as

                (i) Inflammation

                (ii) Resorption (if applicable)

(iii) Injury

(iv) Healing

(v) Integration

c) Impact of animal on device

(i) Device structural integrity

(ii) Device functional integrity

d) Deployment/surgical success, positioning, and overall handling

e) System compatibility, if routinely used with other ancillary devices

f) Imaging characteristics

viii. Conclusions

a) Conformity with controls

b) Success in meeting acceptance criteria

c) Identification of related studies that were conducted or are scheduled to be completed that explain any outstanding issues

8. Indexed Secondary Attachments (raw data and individual test reports)

a. Vendor reports

b. Baseline and interim health examinations

c. Surgery and anesthesia reports

d. Imaging reports

e. Clinical pathology results

f. Electromechanical results

g. Copies of animal medical records

h. Signed and dated Contributing Scientist(s) reports (e.g., interventionalist, surgeon, radiologist, clinical veterinarian, clinical pathologist, pathologist etc.). These reports may need:

i. Images (e.g., explant radiography images, *in situ* photography, gross and histopathology, angiography)

ii. Cinematography

iii. Electrophysiology strips

iv. If applicable and part of the raw data, consider providing case report forms.

Appendix E: Tabulated Summary of Relevant Federal Regulations and Guides (the list below is not intended to be exhaustive)

Topic	Regulatory Citation
GLP Animal Care	21 CFR 58.90
Protocol	21 CFR 58.120 and 58.130
Quality Assurance Unit	21 CFR 58.35
Test and Control Articles	21 CFR 58.105 and 58.107
Records and Reports	21 CFR 58.185, 58.190, and 58.195
Test System	21 CFR 58.3(i)
Federal Animal Biomedical Research Standards	9 CFR Chapter I, Part 3
Housing and Well-Being of Dogs	The care, exercise, and housing of dogs are described in 9 CFR Chapter I, Part 3 Standards, Subpart A. Housing, animal management, and species-specific space recommendations are provided in the National Research Council (NRC) publication, "Guide for the Care and Use of Laboratory Animals,"[4] which is the recommended reference to which metrics are applied by AAALAC and the PHS.
Sanitization and Husbandry	9 CFR Chapter I, Part 3 Standards and in the "Guide for the Care and Use of Laboratory Animals."[4]
Environmental Control of Transportation	9 CFR Chapter I, Part 3 Standards and in the "Guide for the Care and Use of Laboratory Animals"[4]
Animal Identification Systems	Identification of warm-blooded animals (except suckling rodents) is discussed in 21 CFR 58.90, and also, with respect to dogs and cats and all other animals used in research, within 9 CFR Chapter 1, Part 2, Subpart E.
Animal Quarantine and Conditioning	21 CFR 58.90 and in the NRC "Guide for the Care and Use of Laboratory Animals,"[4] Page 110. The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[39] provides similar guidance to European member state facilities.
Social and Environmental Research Standards	9 CFR 3.7 and 3.8, and in the NRC "Guide for the Care and Use of Laboratory Animals,"[4] pages 52-56, 63-65, 82-84.

Appendix F: Additional Resources on Animal Care and Research

1. FDA Guidance for Industry (2007). Good Laboratory Practices - Questions and Answers. (http://www.fda.gov/downloads/ICECI/EnforcementActions/BioresearchMonitoring/ucm133748.pdf)
2. Hasenfuss G (1998). Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovascular Research,* 39(1) 60-76.
3. National Research Council Board on Agriculture Subcommittee on Dog Nutrition. Nutrient Requirements of Dogs, Revised. (1985). Washington, D.C.: National Academy Press. (http://www.nap.edu/openbook.php?record_id=15&page=R1)
4. National Research Council Board on Agriculture and Renewable Resources Subcommittee on Goat Nutrition. Nutrient Requirements of Goats: Angora, Dairy, and Meat Goats in Temperate and Tropical Countries. (1981). Washington, D.C.: National Academy Press.
5. National Research Council Board on Agriculture Subcommittee on Sheep Nutrition. Nutrient Requirements of Sheep. 6th rev. ed. (1985). Washington, D.C. National Academy Press. (http://www.nap.edu/openbook.php?record_id=614&page=R1)
6. National Research Council Board on Agriculture Subcommittee on Nutrient Requirements of Horses. 6th rev. ed. (2007). Washington, D.C. National Academy Press. (http://www.nap.edu/openbook.php?record_id=11653&page=R1)
7. Flecknell P.A. (1996) *Laboratory Animal Anesthesia*, San Diego, CA: Academic Press Inc.
8. Carr, John. 1998. Garth Pig Stockmanship Standards. Iowa, 5M Enterprises, LTD Sheffield, UK. Website with condition images. (http://www.thepigsite.com/stockstds/23/body-condition-scoring/. Accession date 11/14/08)

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[1] United States Department of Agriculture, 9 CFR Parts 1, 2, and 3 (Animal Welfare).

[2] United States Public Health Service (2002). Policy on Humane Care and Use of Laboratory Animals. Office of Laboratory Animal Welfare, National Institutes of Health. Bethesda, MD. (http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf)

[3] United States Government Health Research Extension Act of 1985, Pub. L. 99-158; and US Government Principles for the Utilization of and Care of Vertebrate Animals Used in Testing, Research, and Training. (http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples)

[4] National Research Council Guide for the Care and Use of Laboratory Animals, 8th ed. (2011). Institute of Laboratory Animal Resources Commission on Life Sciences, Washington, D.C. National Academies of Science Press. (http://www.nap.edu/catalog.php?record_id=12910)

[5] The FDA maintains an intergovernmental Memorandum of Understanding (MOU) between NIH, FDA, USDA regarding common areas of regulatory interest in animal care and use. (http://grants.nih.gov/grants/olaw/references/finalmou.htm)

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[7] Ma, C. and J-M Zhang (eds) (2011). Modeling Pain in Laboratory Animals: A Collection of Protocols. 1st Ed. Humana Press: New York.
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[12] An YH and RJ Friedman (eds.). (1999) Animal Models in Orthopaedic Research. CRC Press, LLC., Boca Raton, Fl.
[13] For more information on Pre-Submissions, see the FDA guidance, Requests for Feedback on Medical Device Submissions: The Pre-Submission Program and Meetings with Food and Drug Administration Staff (http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM311176.pdf).
[14] The Interagency Coordinating committee on the Validation of Alternative Methods (ICCVAM) provides many websites for decisions related to animal care and use, refinements, reductions, and replacement of animal models and validated models (http://iccvam.niehs.nih.gov/about/accept.htm).
[15] The European Centre for the Validation of Alternative Methods (ECVAM) is a useful web link to validated European alternative animal models. (http://www.bfr.bund.de/en/european_centre_for_the_validation_of_alternative_methods__ecvam_-4411.html)
[16] The Japanese Convention on the Validation of Alternative Methods is a resource for those animal models and animal welfare items of interest from Japan. (http://jacvam.jp/en/)
[17] The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is a comprehensive web resource for all subjects related to global animal care and use, animal welfare, and animal alternatives. (http://caat.jhsph.edu/)
[18] The National Center for 3Rs is a comprehensive web link for animal care and use and animal refinement and replacement questions. (http://www.nc3rs.org.uk/)
[19] AltTox Forum (sponsored by Proctor and Gamble and The Humane Society of the United States) provides information about validated animal alternatives. (http:// alttox.org/)
[20] Tox Net is an NIH-sponsored website of available literature on alternatives. (http://toxnet.nlm.nih.gov/altbib.html)
[21] Bassert, Joanna M. Medical Records. Chapter 5. IN: McCurnin, Dennis M and Joanna M Bassert (eds). 2010. Clinical Textbook for Veterinary Technicians, 7th edition. Saunders, St. Louis, MO.
[22] Reinhart, V. (2002). *Comfortable quarters for laboratory animals ninth ed*. Washington, D.C. United States Department of Agriculture Animal Welfare Information Center

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[24] Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd edition 2010, Federation of Animal Science Societies (FASS). (http://www.fass.org/page.asp?pageID=216&autotry=true&ULnotkn=true)

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[27] National Research Council Institute for Laboratory Animal Research Committee on Guidelines for the Humane Transportation of Laboratory Animals (2006). Nutrient Requirements of Beef Cattle. 7th rev. ed. Washington, D.C.: National Academy Press. (http://www.nap.edu/openbook.php?isbn=0309069343)

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