Contains Nonbinding Recommendations

Guidance for Industry and FDA Staff

General Considerations for Animal Studies for Cardiovascular Devices

Document issued on: July 29, 2010

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Preface

Public Comment

You may submit written comments and suggestions at any time for Agency consideration to the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Submit electronic comments to www.regulations.gov.

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General Considerations for Animal Studies for Cardiovascular Devices

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I. Introduction

FDA has developed this guidance document to assist industry in designing evaluation strategies for, and reporting the results of, animal studies involving cardiovascular devices, including intra-cardiac devices and devices used in the coronary and peripheral vasculature. 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. We recommend that members of industry and FDA staff who perform or review evaluations of animal studies for cardiovascular devices use this guidance. 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, conduct, and presentation of animal study data intended to demonstrate that the device under study is sufficiently safe for early human experience, while accounting for modern animal care and use strategies. The guidance makes multiple references to pre-existing regulatory requirements involving animal care and use.[1-5] 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 practice under which animal studies are to be performed.[38]

We recommend that you use this guidance to develop and present animal study protocols, methods, and reports that support the safety and performance of cardiovascular devices. We intend to use this guidance to review animal study protocols, methods, data, and reports provided in regulatory submissions to demonstrate the safety and performance of 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 devices intended for use in the human cardiovascular system. Cardiovascular devices encompass an array of product classifications, and many such devices are defined in 21 CFR Subchapter H, Part 870.[1] This classification consists of medical devices intended for intra-cardiac or extra-cardiac use, or for use in the coronary and peripheral circulations. The scope includes the carotid and renal vasculature, but excludes the neuro-vasculature.

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. The recommendations in this document are also consistent with those already published under Title 9 of the US Code and its applicable definitions, regulations, standards, rules of practice, and policies.[2,4,5]

III. Overview

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

- You should follow Good Laboratory Practices (GLP) for all animal studies involving cardiovascular devices that are to be submitted to the Agency.

- The animal model selected for a cardiovascular study should be generally accepted for the study of the device type. That is, there should be a reasonable amount of scientific evidence that the animal model has utility for the study of the product class.

- FDA's primary purpose in asking for an animal study for a particular device is to demonstrate sufficient safety including performance and handling. A secondary objective is to study the efficacy of the device, if applicable. Sometimes the performance of a particular device is intricately linked to its safety, such as for products that provide circulatory support.

- The *in vivo* setting generally provides FDA with 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.

- 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.[37-45]

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

IV. Elements of the Animal Study

FDA recommends that your regulatory submissions include a discussion of each of the following key animal study features:

- introduction, including a rationale for the selection of the particular animal model;
- the study assurances;
- the purpose and objectives of each test protocol;
- the study schedule;
- the methods and procedures utilized in the study;
- the characterization of the test articles and test systems;
- the *in vivo* study results;
- any *ex vivo* test article characterization;
- any *ex vivo* tissue characterization; and
- the relevant study conclusions, including 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 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. FDA believes that 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 the 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 size, including the diameter and length, 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;

- the tortuosity of the vascular bed of interest, and why any size limitations exist as barriers (exclusive of cost) to obtaining the most size-appropriate model; and

- if the device is intended for use in or near the heart, such as a prosthetic heart valve or an endovascular aortic aneurysm graft, the selection of the profile and diameter of the device, and the distance to key cardiac structures such as the coronary ostia and the chordae tendinae.

B. Study Assurances

FDA requires that animal studies comply with animal care and research conduct as detailed in 21 CFR Parts 58.90 and 58.130. Because managerial independence of the Quality Assurance Unit (QAU) is required by FDA regulations (21 CFR 58.35), we expect the final study report to be the report resulting from an independent review of GLP. If it is not clearly stated in the Quality Assurance Statement (QAS) the test report should clarify how the QAU is operationally independent from the study managers. The statement should also include dates of inspection of each aspect of the study schedule.

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. FDA also recommends that you hire an independent auditor so that impartial quality assurance is provided.

C. Study Objectives

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

Recommendations for evaluating specific types of risks are provided below.

1. Performance and Handling

FDA recommends that you consider combining performance and handling test protocols with chronic study protocols. One potential way to accomplish this goal is to develop case report forms for the implant phase of the study as a mechanism for capturing handling, deployment, and implantation information. As part of the study protocol, you should identify all steps required to deliver or implant the device, and develop acceptance criteria for each of the steps. FDA recommends that you apply an objective or subjective rating scale to each acceptance criterion. If the device is delivered 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, 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 steps should be described.

2. Device Safety

a. Mortality and Morbidity

FDA recommends that you fully explain all observed instances of animal illness and death, and that any statements made regarding whether such events are device-related be thoroughly described. Retrospective testimonials or statements made by study directors, their designates, 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 for the purpose of detailing wellness or morbidity, including the development of key assessments for systemic effects of device use. These assessments includepostoperative, interim, and terminal clinical pathology, such as but not limited to serum creatinine; liver enzymes, (e.g., alkaline phosphatase); and electrolytes, (e.g., sodium, potassium and calcium); and hemograms, including white and red blood cell indices.

b. Vascular Safety

Cardiovascular devices can cause mechanical or biologic stresses when used in the vasculature. If your device will be used in or tracked through the

coronary or peripheral vasculature, FDA recommends that you identify key biologic response variables at regional sites, at locations adjacent to the implant site, and along all paths to and from the point of implantation to develop active means of surveying the impact of your device on the vasculature. You may wish to work with pathology experts as part of this approach. Some common biological evaluation parameters include:

- type and quantity of inflammatory components, such as neutrophils, eosinophils, lymphocytes, giant cells, and macrophages;
- the cellularity of the intima, media, and adventitia;
- the presence of mineralization or ossification; and
- injurious events and biological responses such as disruption of the internal or external elastic lamina, perforation, dissection, hemorrhage, fibrin formation, and neointimal proliferation.

c. Downstream and Systemic Effects

FDA recommends that you evaluate whether or not devices placed in the heart and vasculature can have effects remote from the site of placement. If you believe that your device has the potential for this type of risk, you should ensure that your study includes objectives to evaluate downstream tissue for evidence of potential emboli that might be part of the device and delivery system, or thrombi that might originate at the site of the device and progress downstream. 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 endpoints for your study, based on your knowledge of the known risks and predicated outcomes of the device. These endpoints typically include:

- full characterization, implantation, and intermittent examination of device performance and/or animal response;
- explantation of the device;
- full analysis of any explanted tissue;
- preparation of the tissue; and

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

FDA recommends that the QAU be aware of these key scheduling objectives so that interim study monitoring and inspections can be arranged. Because cardiovascular devices may involve some degree of invasiveness and predictable variability in animal survival, FDA believes that 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.[5] FDA believes that the responsible use of animals optimizes the use of all animal tissue and that tissue that is not freshly studied is prone to erroneous interpretation.

E. Characterization of Test and Control Articles

FDA recommends that you fully characterize and account for all test and control articles used in the study. Since investigators may often develop several iterations of the test article prior to clinical study initiation, FDA recommends that pivotal animal studies utilize test articles representing the final clinical design. If the final design was not used, FDA recommends that you provide a rationale for why the final clinical design presents no new risks to the patient compared to the design studied in animals. In addition, FDA recommends that test and control articles be packaged, sterilized, and shipped to the research site in the same manner as would clinical product. Finally, FDA recommends that you 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. FDA recommends that you explain whether any accessory devices used in the animal study are completely separate from the test article, whether they will be marketed together with the test article, and whether the final labeling for the device will include instructions for accessory device selection or use.

G. Test System

FDA recommends that you provide a sufficiently detailed description of the test system so that FDA can make a reasonable assessment of all physical and environmental contributions to the study outcome. 21 CFR 58.3(i) defines *test system* as, "any animal, plant, microorganism, or sub-parts thereof to which the test or control article is administered or added for study. Test system also includes

appropriate groups or components of the system not treated with the test or control articles. For the purpose of this guidance, FDA considers key components of a test system to include 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.

V. Personnel

FDA recommends that each test report contain a section that lists key study personnel. FDA believes that this information is relevant to regulatory review because all applicable regulations require that study personnel are appropriately trained and experienced to properly carry out their duties. [2, 3, 5, 13,15] In addition, FDA believes that the training and expertise of animal study personnel are especially important for cardiovascular devices because many cardiovascular procedures require invasive techniques and a high level of technical proficiency. Because the risks associated with cardiovascular devices may be associated with subclinical or discrete subjective and objective findings, 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 protocol in the interest of humane care, preserve valuable tissue, and to assist in the determination of device associations with any adverse finding.

An animal study to support a cardiovascular device usually begins after a risk analysis, bench testing, and other information, including scientific presentations, publications, or non-anecdotal experiences, have identified the expected risks associated with the device. 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 best practices stated in 21 CFR Part 58 and other federal regulations and policies, we also recommend that you record the qualifications and experience of all personnel engaged in the conduct of animal studies and 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. [2, 4]

In addition to assembling a cadre 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 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 products in the same class of devices. Finally, FDA recommends that you employ veterinary professionals with adequate training and experience to evaluate the facilities, personnel, and methodology that you may wish to contract from other business entities, such as contract animal research or holding facilities.

VI. Facilities

A. Environment

FDA recommends that you follow previously 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.[2-6, 17-20]

While the selection of an appropriate animal model is left to the sponsor, FDA has observed that dogs comprise the bulk of research involving cardiac pacing and models in which heart failure is necessary. FDA has also observed that small ruminants serve as a model for prosthetic heart valves and artificial circulatory support devices, and that small swine represent the majority of other cardiovascular research animals. As a result of these preferences, the recommendations on animal study facilities in the following paragraphs focus on these particular models.

FDA recommends that you consult prior publications for recommendations and regulations involving the housing and well-being of dogs[5-7], swine and small ruminants[8-11]. The referenced regulations and guides address minimum housing and husbandry standards and social and environmental enrichment requirements, and encourage the development of standard operating procedures that address timely and adequate veterinary medical care. FDA believes that following these procedures and allowing animals sufficient access to resources such as food and water receptacles, toys, and clean and species-typical resting surfaces, and providing them with opportunities for postural adjustments, adequate play and exercise, and 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, FDA recommends that the floors, walls, and ceilings of animal holding structures be non-porous in order to permit easy sanitization of surfaces. FDA recommends that there be adequate lighting and light controls to permit periods of normal daylight and opportunities for rest. FDA also recommends the utilization of facilities with appropriate environmental controls for temperature and humidity in order to prevent temperature stress and minimize respiratory infections.[5]

B. Animal Groupings

While 21 CFR Part 58.43 recommends isolation of intra-species test systems, other national policies encourage animal play and contact in groupings in order to meet standards of social enrichment. Regardless of whether your test system consists of one group or multiple groups of animals within a study, FDA recommends that the environmental conditions not interfere with the assessment of the device, and that all animals have access to the same resources. If your protocol allows conspecific housing, FDA recommends that you consider the effects of inter-animal

socialization and tolerance once animals are housed in groups, and that you provide adequate resources such as food, water, and toys in order to prevent bullying and territorial stress.

C. Primary and Secondary Enclosures

Because many cardiovascular 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. FDA also recommends 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 such as small swine, ungulates, and dogs 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 to make transportation less stressful.[12] Transport vehicles should afford the animals environmentally-controlled heating and air conditioning in order to further minimize shipping stress.[2, 5,]

VII. Study Methods and Conduct

FDA recommends that the methods and materials utilized for the use of cardiovascular 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. Because animal systems are imperfect and may often detect new safety concerns, the study documentation should describe when protocol deviations typically can occur or have occurred, and how these new safety concerns have been or will be addressed.

A. Research Controls

Evaluation of device safety prior to human investigational use is based largely on animal studies that provide valid scientific evidence (21 CFR§860.7), 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 makes a distinction between the research controls discussed in this documentand a control article. We consider adequate animal research controls to include but not be limited to anything given to or affecting the test animal in the course of an experiment that would impact the

comparison between the test and control animals. Controls 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. For the purpose of assessing the effect of the test device on the animal and of the animal on the device, FDA considers research controls to not only include materials, but also minimization of confounding factors resulting from unequal or erroneous methods and practices, as these factors can hinder the ability of the investigator to clearly associate adverse or positive outcomes with the device and or its effects.

With this consideration in mind, FDA recommends the use of personnel, consumable equipment, and practices that enable test article-associated outcomes to be clearly understood. Within the context of animal studies, FDA recommends that you consider the use of the following controls:

- provision of physiologic homeostasis, such as adequate thermoregulation, electrolyte, and blood glucose and caloric balance;
- procedures that minimize infection;
- a program to maximize animal wellness through the provision of species-specific social nutritional adequacy and environmental enrichment;
- procedures that standardize the methods for the collection, handling, and shipment of tissue specimens; and
- personnel and practices that demonstrate ample levels of training and experience.

A sample reference of key controls for animal research studies is included as Appendix C.

B. Study Equipment

Given that a cardiovascular 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 [15], FDA recommends that you encourage early and frequent interaction between personnel involved in the planning of the animal study and those who will actually perform the study. FDA believes this dialogue is especially important to ensure that the study facilities have the proper ancillary equipment and supplies for the study.

One common complexity in animal studies for cardiovascular devices is that animals tend to benefit from multiple drug combinations to attain adequate anesthetic depth and control of pain and distress, vascular spasm, infection, and thrombosis. In addition, imaging equipment and personnel may be as advanced as

those found in human interventional suites. Because of this relatively high level of support, FDA recommends that study sponsors, their consultants, and the study director carefully assess the care, maintenance, and knowledge of any contract equipment associated with the conduct of the study.

C. Animal Identification

FDA recommends that animal identification, age, weight, sex, and baseline health be fully characterized.[1-3,] If animals are re-identified for a particular study, you should include a table of information pertaining to animal identification, allocation to study sub-groups, 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 recipient is possible.

D. Animal Quarantine and Conditioning

FDA recommends that you follow the FDA and USDA regulations prescribing that animal facilities adopt and implement standard operating procedures that permit for adequate periods of quarantine and acclimation, as well as a program of socialization.[1,5,] Because cardiovascular studies can be invasive and entail predictable levels of pain or distress, 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, FDA recommends 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 recommend that you follow the Animal Welfare Act, PHS policy, and their applicable regulations and statutes, as these guidelines and regulations have resulted in important changes in the use of environmental and socialization protocols that are routinely implemented to control background stress. FDA believes that following these regulations enhances the opportunity and intensity of observations and can potentially result in other useful findings for the investigators. [1-5]

E. Animal Allocation to Experimental Grouping

When considering the number of animals and the amount of data that can support the safety and performance of a cardiovascular device, FDA recommends balancing the veterinary principles of reduction/replacement/refinement as well as regulatory least burdensome principles, with the goal of using the minimum number of animals necessary to demonstrate adequate safety and performance. FDA believes that this determination can best be made after bench testing is complete and the device iterations are finalized, although we recognize that this is not always feasible. FDA

recommends that the number of animals also be based on the difficulty of the model and whether one or more test article and/or control 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 heart valves can involve such a high degree of expertise and some expected morbidity that a relatively large number of animals may be appropriate, in order to allow the Agency to reasonably interpret device safety. For novel devices or animal models that are expected to be challenging, FDA recommends that you complete key bench tests and conduct a full risk analysis before developing an animal study strategy, so that your studies can include the most comprehensive assessment of device safety and performance. If you wish to discuss your animal study strategy with FDA prior to study initiation, you should provide this information, as it can assist FDA in balancing responsible animal use and least burdensome principles with a best effort to discover appropriate *in vivo* safety. Designing and conducting animal studies prior to obtaining full knowledge of *in vitro* performance or device-related risks may result in the need for additional animal studies if new risks or failure modes are subsequently identified or elevated.

F. Food, Water and Basic Husbandry

FDA recommends that study animals be free of disease and that you provide adequate resources to prevent conditions such as aggression, disparate growth rates, and different patterns of sustenance and healing, and account for species-typical states of wellness. As part of this approach, you should consider previously documented nutrient requirements of commonly utilized animal species for cardiovascular device research.[20-23]

FDA believes that weight loss, which can occur when incisions are made through multiple tissue layers, or when recovery from a procedure is slow, can be challenging to interpret, especially when accompanied by low serum albumin levels. Thus, in order to separate weight loss as a sign of device-associated disease, FDA recommends that you follow 21 CFR§58.90, which requires regular inspection of food and water for contaminants as well as proper nutritional ingredients, and that research facilities have standard operating procedures for the feeding and hydration of animals and for the evaluation of body condition.[1] As part of animal evaluation, you should consider adopting a body scoring paradigm, a number of which have already been developed for a number of species.[33-36] You should ensure that individuals monitor animals to document specifics regarding appetite, thirst, and food and water intake, particularly when animals are pen-housed. Bullying and resource coveting are commonly associated with weight loss due to inadvertent caloric or fluid intake, and if not actively surveyed, an animal or group of animals may present with unexpected weight loss near the conclusion of a study when intervention is not possible. 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.[5]

G. Periods of Observation

In keeping with the provisions for adequate veterinary care that are common to all applicable national regulations, standards, policies, and procedures, [1-5,11, 16] FDA recommends that you monitor study animals at a frequency and intensity that adequately assesses for known risks posed by the device, and that you work with attending veterinary staff at the study facility to develop these monitoring parameters. FDA believes that such monitoring is appropriate not only for humane reasons, but also because well-monitored animals best help FDA to 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 should 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, FDA recommends 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. 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, FDA recommends that you track and record co-variables such as cardiac rhythm,

respiratory rate, and blood pressure on operative records so that FDA can evaluate safety concerns associated with major periods of implant. The timing of insertion or deployment of the device and/or contrast or other device-associated materials of use should be noted on the anesthetic and/or operative records.

2. Chronic Studies

a. Post-Operative Period

In FDA's experience, the post-implant or post-surgical period for most cardiovascular studies is frequently one of intensive monitoring. 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. [5, 25-32] You should capture physiological information similar in quality to that obtained in human intensive care and recovery areas, so that any early success and failures deaths can be well described clinically as well as by necropsy information if, for example, it is necessary to euthanize an animal for humane considerations. 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 recommend that you collect animal weight information on a weekly basis to establish any weight loss as early as possible. You should consider inclusion of body scoring to monitor conditions such as ascites of cardiogenic origin, especially when cardiac output is a parameter of interest in the study, as these animals may be losing body mass while still preserving weight as fluid.

If your study involves the collection of clinical chemistry data or more advanced diagnostics, FDA recommends that you develop standard operating procedures that prescribe a method of chemical restraint that does not interfere with the device. In FDA's 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 collection and processing of tissue. This section of the protocol should include the following information:

- methods for end-period examination;
- whether or not in-life radiographic analysis of blood flow or device positioning is needed;
- methods for establishing end weight; and
- anticoagulation, euthanasia, and tissue harvest methods, so that we can determine whether all observations made during necropsy and tissue preparation are related or unrelated to the method of euthanasia or harvest.

d. Necropsy and Post-Mortem Evaluation

FDA recommends that you include a comprehensive systematic necropsy in your study, as the resulting information can help FDA to determine whether observed adverse events are device-related. You should support any statements regarding whether any adverse outcomes are device-related with appropriate evidence from the necropsy report and from in-life observations.

H. Post-Mortem Imaging and Assessment Methods

1. Explant Radiography

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. For devices that are implanted in coronary arteries or intra-cardiac locations such as coronary artery stents or prosthetic heart valves, you should consider the use of device radiography in explanted whole hearts.

2. Scanning Electron Microscopy (SEM)

FDA recommends the use of SEM for characterizing the surface behavior of devices implanted in animals and also for the characterization of endothelialization of coronary and peripheral stents. We recommend that your regulatory submissions include representation of best- and worst-case outcomes, so that FDA can better appreciate the range of outcomes of your study.

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, FDA recommends that you seek the expertise of board-certified veterinary or clinical pathologists when developing and executing methods for preparing explanted cardiovascular 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 vascular and peri-vascular tissue. We recommend that you consider the inclusion of the following assessments in your evaluation:

- endothelialization;
- mural injury;
- inflammation;
- vascularization;
- intimal fibrin;
- intimal and medial smooth muscle cell proliferation;
- adventitial fibrosis; and
- the integrity of the internal and external elastic laminae.

FDA recommends that you report the tools and methods used to extract the tissue and the methods of fixation, cutting, and staining. The reports should also include diagrams indicating the location of sectioned vessels or cardiac structures, the sectioning methods, and the magnification of each section image. When discussing the study results, you should include well-marked high resolution color images, each indicating the animal number, study group, tissue section, magnification, and other important identifiers.

4. Local and Downstream Tissue Assessment

FDA believes that most cardiovascular 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 downstream pressure necrosis and inflammation. If your risk analysis identifies this potential risk for your device, FDA recommends that your pathologic study include systematic descriptive evaluation of downstream 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. As part of your evaluation strategy, you should consider whether retrograde embolization is also possible. For example, venous congestion may occur with devices placed in the coronary sinus, and renal congestion may occur due to thrombus generated from inferior vena cava filter implantation.

VIII. Records and Reports

FDA recommends that you prepare the records and reports for your animal studies such that FDA can most efficiently evaluate device safety and performance. You should include the animal recordings and key study attributes as appendices to the study protocol, and organize their format and content with the goals of explaining all study outcomes and minimizing ambiguity in how contract and subordinate research personnel interpret their responsibilities. FDA recommends 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, so that the inter-observational differences between study subjects may be established as part of the conformity to the test protocol.

IX. Preparation of Regulatory Submissions

When preparing regulatory submissions, including IDE, 510(k), and PMA submissions, FDA recommends that you include all information collected as part of your animal studies. FDA also recommends that your submission include a summary that provides an overview of all the animal studies you conducted. This summary should discuss the number of 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 quality systems in place during the study; and
- how the quality systems maintained independence and impartiality in the inspection of the data and the reporting of the results.

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

FDA recommends 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 test group allocation;
- study duration;
- the device design iteration used; and
- a summary of study outcomes.

In addition, you should provide an attachment for each animal study that includes study details, including individual scientific reports, 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 within the submission.

When compiling more than one study into a group of attachments, FDA recommends that you do so in the order in which the studies were performed so that FDA 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 the animal study section of regulatory submissions is provided in Appendix D. Finally, FDA recommends that your submission include the animal study information in an electronic bookmarked file or similar format, as such a format can assist FDA with the animal study review.

In addition to these considerations, FDA recommends 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

AAALAC: International Association for Assessment and Accreditation of Laboratory Animal Care

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

CLIA: Clinical Laboratory Improvements Act

CFR: Code of Federal Regulations

FDA: United States Food and Drug Administration

GLP: Good Laboratory Practices

IACUC: Institutional Animal Care and Use Committee

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 Animal Studies in the Cardiovascular System

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 or the CDRH Medical Device Report (MDR) database (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmdr/search.CFM)

    a. If yes, go to step 2.

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

2. Have all of the evaluable risks been tested on the benchtop using the final design iteration?

    a. If yes, go to Step 3.

    b. If no, we recommend completion of bench testing with the final design before proceeding to step 3, although such an approach may not always be possible.

3. 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 4.

    b. If no, have you assessed the anatomy and physiology of commonly utilized laboratory animal species (such as small hoofed stock, dogs, and primates) for size and procedural approach features?

        i. If yes, and you can identify an animal model that would work, go to Step 4.

        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. 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 Unites 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 4 or Step 3.b.ii.

4. 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 and time points, and proceed to Step 5.

    b. If no, FDA recommends that you use the time points reported for similar devices, and proceed to Step 5.

5. 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 6.

    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 feasibility animal studies to evaluate this issue prior to conducting pivotal animal studies and before proceeding to Step 6.

6. 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-IDE that includes a proposal for your pivotal GLP animal studies. This proposal should detail all methods of assessment for identified risks that may be observed dynamically in life and with gross and histopathology, and include any specific questions for which you would like FDA input. More information regarding the pre-IDE process is available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/InvestigationalDeviceExemptionIDE/ucm046164.htm#pre_ide.

Appendix C: Recommended Animal Study Research Controls to Consider

- A determination of satisfactory source animal health, based on veterinary examinations
- Methods that permit a knowledge of food and water purity and nutritional adequacy for the species under study, such as:
    - regularly scheduled interim weight measurements
    - provision of adequate number of feeders in pen-housed animals
    - standard operating procedures to screen for food and water contaminants
    - 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 peri-procedural
- Use of an acclimation period after the source animals arrive at the test facility, such as 7 to10 days
- Appropriate baseline assessments of animal health and behavior
- Proper aseptic surgical technique, and monitoring and intervention to control unintended infections
- Practices and procedures that ensure the animal facility staff are providing adequate sanitation and air conditioning to prevent unintended injury and infection
- 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
- Practices ensuring that all experimental equipment is calibrated and maintained according to accepted standard operating procedures
- Practices ensuring that there is adequate personnel and staffing to ensure 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
- Appropriate monitoring and timely postoperative monitoring and intervention to detect, control, and report common cardiovascular outcomes such as spasms, arrhythmias, pain, and distress

- Practices ensuring that transportation and shipping stress is minimized when moving peri-procedural animals to remote holding sites

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

- Practices that encourage adequate and timely intervention to obtain necropsies when animals die unexpectedly

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

- Practices ensuring that the test system, test article, control article, and all specimens or data collected from the test system are properly identified and that their identity is not confused

Appendix D: Sample Organization of Animal Study Test Reports

1. Report number
    a. Institutional protocol number
    b. Study number(s)
    c. Test protocol number(s)
2. Title of the report
3. Contact information
    a. Sponsor
    b. Sponsor representative
    c. Test facility name(s)
    d. Study director
    e. Quality Assurance director
4. Final report signature page
    a. Study Director's signature
    b. Quality Assurance signature
    c. Copy of Institutional Animal Care and Use Committee (IACUC) protocol, signed by the IACUC chairperson and attending veterinarian
5. Executive summary
    a. Overview of animal study
        i. Objective of the study
        ii. Acceptance criteria
        iii. Rationale for selection or exclusion of animals, including supporting discussion and rationale if the proposed animal model could not be used
        iv. Characterization of test and control articles

a) Design iteration of device used

b) Referenced serial or model numbers

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

vi. Brief overview of results

a) Morbidity/mortality

(i) Gross necropsy information

(ii) *In situ* photography

(iii) Descriptive findings

b) Biologic response to the device

(i) Inflammation

(ii) Endothelialization

(iii) Injury

(iv) Fibrin and/or thrombin formation

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 cardiovascular devices

f) Imaging characteristics

vii. 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

6. 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 chemistry results

    f. Electromechanical results

    g. Explant radiography images

    h. *In situ* photography images

    i. Principal investigator report

    j. Signed descriptive pathology report, with accompanying images

    k. Signed descriptive histopathology report, with accompanying images and raw data

    l. Signed interventionalist or surgeon's reports on device performance, with accompanying case report forms

    m. Signed attending veterinary statement

Appendix E: Tabulated Summary of Relevant Federal Regulations

Topic	Regulatory Citation	Applicable Guidance Section
GLP Animal Care	21 CFR 58.90	Section III and Section IV, Part B
Protocol	21 CFR 58.130	Section III
Quality Assurance Unit	21 CFR 58.35	Section IV, Parts B and D, and Section V
Test and Control Articles	21 CFR 58.105 and 58.107.	Section IV, Part E
Test System	21 CFR58.3(i)	Section IV, Parts A, F and G
Federal Animal Biomedical Research Standards	9 CFR Chapter 1, Part 3 Standards § 3.1	Section VI, Part A
Housing and Well-Being of Dogs	The care, exercise, and housing of dogs are described in 9 CFR Chapter 1, Part 3 Standards. Housing, animal management, and species-specific space requirements are generally prescribed as performance standards in the NRC publication Guide for the Care and Use of Laboratory Research Animals which is the recommended reference to which metrics are applied by AAALAC and the PHS.	Section VI, Part A
Sanitization and Husbandry	9 CFR Chapter 1, Part 3 Standards § 3.11, 3.31 and in The Guide for the Care and Use of Laboratory Animals.[6]	Section VI, Part A
Environmental Control of Transportation	9 CFR Chapter 1, Part 3 Standards §3.5.	Section VI, Part D
Animal Identification Systems	Identification of animals is discussed in 21 CFR, 58.90, and with respect to dogs and cats used in research, within 9 CFR, Chapter 1, Part 2 Regulations §2.9.	Section VII, Part C
Animal Quarantine and Conditioning	21 CFR 58.9 and in the NRC Guide for the Care and Use of Animals[6],	Section VII, Part D and E

	Page 58. The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[41] provides similar guidance to Western European member state facilities.	
Social and Environmental Research Standards	Title 9, Chapter 1, Part 3 Standards §3.7 and 3.8, and in the NRC Guide for the Care and Use of Laboratory Animals[6], page 37.	Section VII, Part E

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38. 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)

39. The Interagency Coordinating committee on the Validationof 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.)

40. The European Convention on the Validation of Alternative Methods is a useful web link to validated European alternative animal models. (http://ecvam.jrc.it/)

41. 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/)

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