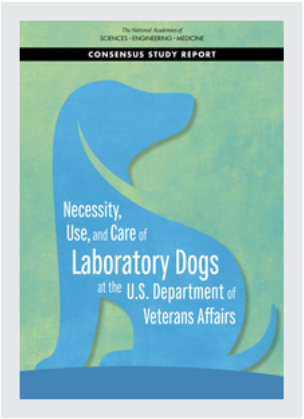

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Necessity, Use, and Care of Laboratory Dogs at the U.S. Department of Veterans Affairs

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Institute for Laboratory Animal Research

Division on Earth and Life Studies

Board on Health Sciences Policy

Health and Medicine Division

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COMMITTEE ON ASSESSMENT OF THE USE AND CARE OF DOGS IN BIOMEDICAL RESEARCH FUNDED BY OR CONDUCTED AT THE U.S. DEPARTMENT OF VETERANS AFFAIRS

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This Consensus Study Report was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published report as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

We thank the following individuals for their review of this report:

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[1] National Academy of Medicine.

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations of this report nor did they see the final draft before its release. The review of this report was overseen by **STEVE BARTHOLD, NAM,** University of California, Davis, and **ELI ADASHI, NAM,** Brown University. They were responsible for making certain that an independent examination of this report was carried out in accordance with the standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the authoring committee and the National Academies.

Preface

This Consensus Study Report represents the culmination of almost 2 years of hard work and dedication of an exceptionally diverse committee of professionals, including industry and academic scientists, physicians, veterinarians, lawyers, and bioethicists. Initially we thought it would be fairly straightforward to answer the primary question, "whether dogs are or will continue to be necessary for any type of biomedical research directly related to the VA's [U.S. Department of Veterans Affairs'] mission." We heard from panels of experts and individual experts, conducted independent data analysis, and were addressed by the senior leadership of the VA's research program. Committee subgroups also visited two VA research facilities. Based on those collective experiences, we initially believed that it would be possible to reach conclusions and make recommendations that all members of the committee could support.

What we all learned is that while facts are always facts, the emphasis that each individual places on each fact and the interpretation of a collection of facts leading to conclusions were widely disparate within this group. The differences seemed dependent on the discipline, each committee member's personal and professional experiences and values, the prevailing attitude of the member's usual constituency, and other, undefined factors. Despite sincere efforts by all to reach consensus, it was not possible. We believe the readers of this report will recognize the intellectual and professional honesty that went into both the majority and the minority conclusions and recommendations.

We want to thank all of the committee members and the experts who generously gave their time and expertise. Additionally, we wish to thank the entire staff at the National Academies of Sciences, Engineering, and Medicine for their tireless efforts to forge a path for us, their outstanding research ability, and their timeliness. Special recognition goes to our study director, Rebecca English, for her calming influence and leadership. Camilla Yandoc Ables and Jenna Briscoe were similarly indispensable in facilitating our requests and requirements for more and more data min-

ing. Keiona Jones and Alex Repace were masters of getting everyone where they needed to be, every time, with all of the support that was needed. The National Academies Research Center also provided invaluable research support, with special thanks to Jorge Mendoza-Torres.

Sincerely,
Rhonda Cornum, *Chair*
W. Ron DeHaven, *Vice Chair*
Committee on Assessment of the Use and Care of Dogs in Biomedical Research
Funded by or Conducted at the U.S. Department of Veterans Affairs

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Summary

Dogs were used in biomedical research as early as the 1st century CE and continue to be used as laboratory animals to the present day, due to their anatomical and physiological similarities with humans, and likely because of their ubiquity and comfort around humans. Today, many public and private institutions in the United States use animals for biomedical research purposes, but over the years the use of dogs has declined. Although the use of animals in biomedical research is regulated by federal laws and is subject to institutional oversight to ensure the humane use and care of laboratory animals, this practice remains contentious and a focus of intense public scrutiny, particularly in the case of dogs.

STUDY ORIGIN

At the request of the U.S. Department of Veterans Affairs (VA), the National Academies of Sciences, Engineering, and Medicine (the National Academies) convened an ad hoc committee to conduct an independent assessment of whether dogs are or will continue to be necessary for biomedical research directly related to the VA's mission. The committee addressed the care and use of laboratory-housed dogs but not of privately-owned companion dogs enrolled in clinical trials. The entire charge to the committee is shown in Box S-1. Note that non-human primates were excluded from the committee's consideration according to the Statement of Task.

To address the study's Statement of Task (see Chapter 1), the National Academies appointed a 16-member committee with expertise in laboratory animal veterinary medicine, biomedical science, medicine, translational research, animal welfare science, animal behavior, animal welfare regulation, alternatives development, bioethics, and animal law.

As one component of its effort to understand the procedures governing animal care and use at the VA and the scientific justification for undertaking research in laboratory dogs, the committee reviewed 14 animal component of research protocol forms associated with current or recent past studies proposing the use of laboratory dogs, as well as additional background materials provided by the VA. The committee supplemented information from expert panels with a comprehensive

BOX S-1
Statement of Task

In response to a request from the U.S. Department of Veterans Affairs (VA), the National Academies of Sciences, Engineering, and Medicine will appoint an ad hoc committee to review the care and use of dogs in biomedical research funded by the VA or carried out in VA facilities, regardless of funding source, for the purpose of advancing science and the understanding of how diseases affect the health of veterans. Specifically, the committee will write a report to address the following:

1. Explore recent past, current, and anticipated research questions directly related to the VA's mission to determine if dogs [rather than non-rodent (excluding non-human primates) or rodent species or non-animal alternatives] are or will continue to be necessary for relevant basic and translational research. The committee will:
   a. Make a determination as to whether dogs are or will continue to be necessary for any type of biomedical research directly related to the VA's mission. If it is determined that they are necessary, describe the unique physiological and other characteristics of dogs that currently make it the necessary animal model for use in these types of research;
   b. Provide recommendations for any new or revised scientific parameters to guide how and when to use dogs for biomedical research rather than non-rodent (excluding non-human primates) or rodent species or non-animal alternatives; and
   c. Explore contemporary and anticipated future alternatives to the use of dogs in this research and determine how these could be part of a strategy to develop and/or use alternatives in support of the agency's mission.
2. Identify ethical considerations, regulatory requirements, and currently accepted standards for the care, use, and welfare of dogs in biomedical research, and make recommendations to enhance their well-being while achieving the research objectives.
3. Determine whether the VA's current review and oversight practices meet the standards, requirements, and recommendations identified above, and make a separate determination as to whether changes in VA practices are recommended.

review of the current scientific literature regarding clinical questions related to the VA's mission that have traditionally used dogs, taking into account both animal and non-animal alternatives to laboratory dog use (see the literature search parameters in Appendix A).

LEGAL, SOCIAL, AND ETHICAL CONSIDERATIONS

Conclusion 2-1: Based on the documentation provided by the U.S. Department of Veterans Affairs (VA) and other organizations (e.g., confirmation of AAALAC International accreditation) as well as site visits, the committee concludes that the VA's biomedical research programs involving laboratory dogs appear to adhere to all relevant policies surrounding animal research.

There is evidence that dogs were the first animal to be domesticated, deriving from the grey wolf at least 14,200 and perhaps as long as 36,000 years ago.[1] Unlike other domesticated species such as pigs and cows, which were primarily selected for traits related to food production, the ancestors of dogs were initially selected for their behaviors.[2] The ancient bonding relationship

[1] Ovodov, N. K., S. J. Crockford, Y. V. Kuzmin, T. F. G. Higham, G. W. L. Hodgins, and J. van der Plicht. 2011. 33,000 year-old "incipient" dog from the Altai Mountains of Siberia. *PLOS ONE* 6(7):e22821.

[2] Serpell, J. A., and D. L. Duffy. 2014. Dog breeds and their behavior. In A. Horowitz (ed.), *Domestic dog cognition and behavior*. Berlin, Heidelberg: Springer-Verlag. Pp. 31–57.

between *Homo sapiens* and *Canis lupus familiaris*, which is manifested in the dog's DNA, is arguably unique. One feature of this relationship meriting reflection is that the docility of dogs, along with their willingness to obey commands and trust humans, makes them easier to work with as laboratory subjects than other large animals. Today, dogs are currently seen in the United States as service, therapy, working, and companion animals—which is likely one driver of public resistance to the use of dogs as laboratory animals.

The preference for dogs can also be viewed as prejudicial. That is, insofar as honoring such a preference would substitute other species for dogs, it discounts the welfare and interests of other species merely because many individuals prefer dogs. Most accounts of animal ethics reject "speciesism" (i.e., the notion that it is acceptable to discriminate against some animals based on their species membership), or at least they place strong constraints on it. The moral relevance of the human–dog unique relationship is highly debatable. According to prevailing accounts of animal ethics in the scholarly literature, there is no basis for discounting the interests and welfare of animals like sheep or pigs relative to dogs.

A condition stipulated by some ethicists is the notion that no experiment should entail unnecessary harm, where necessity is dictated by the purpose of the experiment. On this view, using a species that would experience greater welfare loss for a given research objective would count as unnecessary harm. Rejecting speciesism does not logically entail that all animals should be treated exactly the same. Different animals require different things to flourish. Some animals need certain kinds of bedding, others need certain forms of mental stimulation, and others need space to roam. A rejection of speciesism entails that equal consideration be given to the equivalent interests of different animals and members of different species.

Conclusion 2-2: Many people have a unique relationship with dogs. This relationship stems from tens of thousands of years of joint history and the cultural value of the role of dogs as service, military, law enforcement, and working partners as well as companion animals. This cultural preference for dogs is not universal, nor does it necessarily constitute a reliable guide to ethical action. The majority of the committee concludes that it is valid to consider the societal preference for dogs only in situations where expected burden for substitute species is anticipated to be equivalent to that projected for the laboratory dog.

DETERMINING THE NECESSITY OF LABORATORY DOGS IN BIOMEDICAL RESEARCH FUNDED BY OR CONDUCTED AT THE VA

The committee considered dog use in 10 biomedical research fields related to the VA's mission (see Chapter 3). Cardiovascular disease, spinal cord injury, and imaging are areas of current VA biomedical research using laboratory dogs;[3] diabetes, narcolepsy, osteoarthritis and chronic pain, and experimental pharmacology and toxicology are areas of recent past VA research; and the committee identified three areas considered to be potential candidates for future VA research using (laboratory or companion) dogs—cancer, infectious disease, and Alzheimer's disease. It would not have been feasible for this committee to cover all possible research areas of current or future interest to the VA, and the absence of a particular field from this report should not be taken as a determination regarding the necessity of dog use in that field.

[3] A list of currently active studies in the VA biomedical portfolio that use dogs is available online at https://www.research.va.gov/programs/animal_research/canine_research/current_research.cfm (accessed December 10, 2019).

Conclusion 3-1: The laboratory dog is scientifically necessary for only a few areas of current U.S. Department of Veterans Affairs (VA) biomedical research. Based on the request from the VA to review areas of research from 2016 onward, the committee concludes that laboratory dogs currently remain scientifically necessary in these areas of active biomedical research at the VA:

- *mechanistic insights of premature ventricular contraction-induced cardiomyopathy;*
- *autonomic nerve activity and cardiac arrhythmias;*
- *cardiovascular disease requiring functional modeling of the human Purkinje system; and*
- *development and testing of implantable devices to stimulate respiration and cough in spinal cord injury.*

Laboratory dogs are no longer the preferred model for studies of diabetes or narcolepsy, for most imaging studies, or for primary pharmacological research. Responsibility lies with the principal investigator, scientific review committee, and institutional animal care and use committee to know the literature and accurately determine whether the laboratory dog is still the best model for any particular study.

Conclusion 3-2: A potential new approach or treatment may be developed that, for biological reasons, can be tested only in dogs. As yet unknown, new, or reemerging diseases or disorders may not be reproducible in non-dog models and could require limited use of laboratory dogs to advance their prevention, treatment, or control. Conversely, alternatives may develop in the future that would make the laboratory dog unnecessary.

Conclusion 3-3: The U.S. Department of Veterans Affairs (VA) has an opportunity to expand the study of companion dogs in clinical trials. Companion dogs experience many of the same naturally occurring diseases as humans and stand to benefit from the results of the research in which they participate. Companion dogs are promising models for a range of disorders, including obesity, diabetes, infectious disease, Alzheimer's disease, osteoarthritis, hereditary glaucoma, cardiomyopathy, thoracic spinal cord injury, and cancer. While companion dog clinical trials can be challenging to conduct due, in part, to the financial and time costs of collecting an appropriate population of companion animals for a particular trial, these studies are possible and deserve priority consideration by VA researchers and leaders.

Conclusion 3-4: The committee was not able to fully evaluate the U.S. Department of Veterans Affairs' (VA's) scientific review process for animal research protocols based on the documents provided by the VA, but the committee's analysis of the animal component of research protocol (ACORP) forms revealed deficiencies in the justification for using dogs instead of other species and for the number of dogs used. The ACORP analysis also revealed instances where the investigators did not adequately explain the relevance of the study to veterans' health.

Conclusion 3-5: Principal investigators frequently cited previous experience with and historical data in dog models as primary justifications for using laboratory dogs. These justifications are insufficient alone and constitute a form of circular reasoning that perpetuates the use of laboratory dogs without adequate examination of alternatives.

Conclusion 3-6: The committee notes that certain protocols would have benefited from consultation with veterinary specialists (cardiologists, anesthesiologists, and animal behaviorists) to address animal welfare issues stemming from the performance of multiple

surgeries and multiple sedations or anesthesia on individual dogs and to inform the choice of anesthetizing agents.

Recommendations 1, 2, and 3, if adopted and enforced, would become part of the culture and process of scientific and ethical review at the VA. Recommendations 1 and 2 would create the expectation that principal investigators consider, early in the study proposal process, all possible alternatives (non-animal or animal) and the relative harm the proposed study would bring to the candidate subjects. Scientific review committees and institutional animal care and use committees (IACUCs) would be conducting simultaneous reviews of the analysis of harm and benefit, such that all three parties—principal investigator, scientific review committee, and IACUC—would develop an agreed-upon understanding of "scientific necessity," reconcile any differences of perspective related to the proposed study, and generally pool accountability for decisions related to the use of laboratory dogs.

Recommendation 1: Adopt an expanded set of criteria for determining when it is scientifically necessary to use laboratory dogs in biomedical research funded by or conducted at the U.S. Department of Veterans Affairs (VA).

In order to conduct biomedical research that will lead to meaningful outcomes to support improved health of veterans, the VA should adopt an expanded set of criteria for determining if the use of laboratory dogs is scientifically necessary:[4]

1. **The scientific question and the knowledge anticipated will advance understanding or medical practices related to veterans' health;**
2. **Based on unique physiological and other characteristics, there is no alternative to the laboratory dog that will yield scientifically valid results that meet proposed study objectives;**
3. **The anticipated harms experienced by the laboratory dog are outweighed by the potential benefits for veterans; and**
4. **Both the scientific review committee and institutional animal care and use committee have provided written statements attesting that the laboratory dog is the only species that can yield scientifically valid results.**

After reaching agreement on Conclusion 3-1 and Recommendation 1, the committee found itself at an impasse. Ten committee members,[5] a majority, believed that according to the Statement of Task their job was not done and that a second recommendation linked to Recommendation 1 was warranted, while five committee members[6] were equally convinced that this second recommendation would not be in keeping with the Statement of Task and thus should not be included in the report. The differing opinions of the two groups turn on the meaning of three specific sentences in the Statement of Task—and, in particular, on the meaning of one word—"necessary"—that appears multiple times in those sentences.

To understand the disagreement, some background is useful. Throughout the study process, the committee debated at great length how scientific, legal, ethical, and social considerations factor into the determination of the laboratory dog's necessity in VA biomedical research. In March 2018,

[4] Text was modified after the release of the prepublication report to the sponsor to clarify that some of the criteria in Recommendation 1 are not new to the VA. The committee intends for the criteria, old and new, to be applied as a complete set.

[5] W. Ron DeHaven (*Vice Chair*), Joan Hendricks, Jonathan Kimmelman, Lewis Kinter, Nancy Marks, Christian Newcomer, William Potter, David Powell, Margaret Riley, and Rodney White.

[6] Rhonda Cornum (*Chair*), Donna Arnett, Warren Casey, Chris Green, and Sarah Lathrop.

prior to the VA's request that the National Academies undertake this study, the federal government enacted new restrictions on the VA's use of laboratory dogs, mandating that no federal funds, "may be used to conduct research using canines unless: the scientific objectives of the study can only be met by research with canines."[7] Section 254 of the Consolidated Appropriations Act of 2018 further required the Secretary of the VA to "directly approve" any such studies, and to submit to the U.S. Congress within 180 days, "a detailed report outlining under what circumstances canine research may be needed if there are no other alternatives."[8]

In December 2019, as this committee neared the end of its deliberative process, the Further Consolidated Appropriations Act of 2020 was enacted into law. This legislation reiterated the language from the 2018 Act and expanded it to include cats and non-human primates.[9] The 2020 Act also added a new requirement that such scientific objectives must be "directly related to an illness or injury that is combat-related."[10] Furthermore, the 2020 legislation now requires the Secretary of the VA to submit a report to the U.S. Congress for any such approved research, "not later than 30 days before the commencement of such research."[11] That report must describe the nature of the research and include "the justification for the determination of the Secretary that the scientific objectives of such research could only be met using canines, felines, or non-human primates."[12]

In considering the Statement of Task in the context of this legislation, the members of the majority and minority disagreed about the scientific and ethical implications of this legislation and about its relevance to the committee's recommendations to the VA. Chapter 3 contains a description of the areas of disagreement. To sum up, the disagreement between the majority and minority over Recommendation 2 is essentially a disagreement about whether that recommendation comports with the Statement of Task. The majority, taking a broad view of the meaning of "necessary," believes it does. The minority, holding a more restricted view of the meaning of "necessary," believes it does not. The practical effect of that definitional disagreement is that the majority believes that the interests of other laboratory animals than the dog must be taken into consideration when determining the necessity of research on laboratory dogs, while the minority believes that the question the committee was asked dealt not with other research animals but only with laboratory dogs.

Recommendation 2: Adopt an expanded set of criteria for determining when to use laboratory dogs in the U.S. Department of Veterans Affairs' (VA's) biomedical research when the dog is not scientifically necessary.[13,14]

[7] Consolidated Appropriations Act of 2018, Sec. 254, p. 825 (U.S. Congress, 2018).

[8] Id.

[9] Further Consolidated Appropriations Act of 2020, Sec. 249(a)(b), pp. 665–666 (U.S. Congress, 2019).

[10] Id., p. 666.

[11] Id.

[12] Id.

[13] Five committee members (Rhonda Cornum [*Chair*], Donna Arnett, Warren Casey, Chris Green, and Sarah Lathrop) dissent to Recommendation 2. The dissenters acknowledged the English dictionary definition of "necessary" ("required to be achieved, or essential") as outlined in the Statement of Task to recommend to the VA when the laboratory dog was necessary in biomedical research (i.e., the dog is the only model that will yield scientific results directly related to veterans' health). This is exactly what federal law currently directs. As the Statement of Task did not request an evaluation of other animal models, the dissenters conclude the majority's Recommendation 2 strays beyond the Statement of Task. Additionally, the five committee members argue that a broader ethical framework that is responsive to the public's perception of animal research be considered, especially given that research conducted by the VA is publicly funded.

[14] Text was modified after the release of the prepublication report to the sponsor to clarify that some of the criteria in Recommendation 2 are not new to the VA. The committee intends for the criteria, old and new, to be applied as a complete set.

In order to conduct biomedical research that will lead to meaningful outcomes to support improved health of veterans, the following criteria should be met before approving the use of laboratory dogs when other animal models are also scientifically appropriate:

1. **The scientific question and the knowledge expected to be gained will advance understanding or medical practices related to veterans' health;**
2. **The research objectives cannot be adequately addressed using new approach methodologies or ethically using human subjects or companion animals;**
3. **Where multiple species [excluding non-human primates], including the laboratory dog, can be used to adequately answer the scientific question, the non-primate species that will incur the fewest burdens should be selected. If the species that will incur the fewest burdens cannot be selected for any reason, including legal and/or funding restrictions (e.g., the laboratory dog), the VA cannot ethically proceed and should consider forgoing the research; and**
4. **The expected harms experienced by the selected animals are sufficiently outweighed by the expected benefits for veterans. Both the institutional animal care and use committee and the VA's central office ethics review should concur in this assessment.**

Recommendation 3: Improve biomedical research protocols and review processes, and track the impact of research.

The U.S. Department of Veterans Affairs (VA) should enhance its scientific and ethical review process so that it better integrates the assessment of harm and burden with assessments of value and impact associated with biomedical research using laboratory dogs. There should be an explicit and strong connection between scientific review and institutional animal care and use committee (IACUC) consideration so that all reviewers understand the study objectives, harm–benefit assessment, and anticipated value and impact of the study on human health. The VA should focus efforts on improving the following areas:

- **<u>Protocol Development</u>. Specifically, the VA should implement measures to ensure that:**
  - **The principal investigator starts prior to submission to a funding agency to:**
    - **Develop the biomedical research question and fully describe its value to the VA's mission, veterans, and the nation;**
    - **Engage with an independent literature research group to ensure thorough and transparent evaluation of possible new approach methodology (NAM) alternatives (discussed in Chapter 4);**
    - **Consult with the attending veterinarian to determine whether the requisite veterinary expertise is present in the VA. If additional expertise is needed, the VA's principal investigator should be supported in engaging with veterinary specialists outside the VA to develop protocols and refine procedures necessary to meet study objectives. Examples include newer imaging techniques to measure anatomical and functional parameters of tissues; minimally invasive surgical and interventional radiographic techniques for device placement; and contemporary pain assessment and relief, including current measures of inappetence, weight loss, and other clinical parameters;**
    - **Engage with independent statisticians to ensure appropriate study design and statistical power analysis; and**
    - **Submit the research protocol to funding agency (the VA or other) and IACUC simultaneously.**

- **Protocol Development and Review Processes. Specifically, the VA should:**
  - **Emphasize the replacement of laboratory dogs and the refinement of procedures and techniques over a reduction in animal numbers in order to reduce the burden on individual dogs, even if more animals (including alternative species) will be used;**
  - **Improve literature searches for alternatives to laboratory dogs. The VA should fund an independent party to conduct literature searches designed to yield objective, independent analyses of the need to perform proposed research in laboratory dogs versus alternative animal models, NAMs, humans, or human tissues; and**
  - **Engage with board-certified and other experts in canine medicine and research to review research goals and ensure optimal study design, including estimates of the sample size needed to ensure adequate statistical power. Consider spontaneous clinical conditions of relevance and the possibility of clinical trials in companion dogs to complement or replace laboratory dog studies.**
- **Track Impact of Research. Specifically, the VA should:**
  - **Establish a mechanism for tracking the impact and translation of research using dogs. Such a retrospective reporting mechanism should use objective and state-of-the-art methods (e.g., bibliometrics or citation in regulatory documents and patents) to track the relationship between dog experiments and translated interventions for veterans. Such performance assessment should be required to establish and, if need be, correct risk–benefit and welfare assessments used in the authorization of research.**
  - **Take steps to encourage the prospective registration of all studies involving laboratory dogs.**

ALTERNATIVES TO THE USE OF LABORATORY DOGS

Shifting research to an alternative model (animal or non-animal, including humans) will require successfully addressing those factors that favor the continued use of laboratory dogs. The VA has an opportunity to become a premier biomedical research entity engaging formally with veterinary expertise, both to enhance the experience of laboratory dogs and to conduct clinical trials in companion dogs, using companion dog studies to replace laboratory dog research wherever possible. Accomplishing this goal will require the VA to do the following: (1) engage with experts in canine medicine and research to optimize both clinical methods and research goals, (2) collaborate with researchers conducting clinical trials in companion dogs to identify or develop trials to benefit veterans, and (3) participate in efforts to develop a registry connecting human research needs with companion dog clinical trials.

Conclusion 4-1: The use of companion dogs in biomedical research aimed at benefiting both dogs and humans is a preferred alternative to the use of laboratory dogs. Companion dogs experience many of the same naturally occurring diseases as humans and stand to benefit from the results of the research in which they participate. Established areas of clinical companion dog research with relevance to preclinical studies in veterans include cancer and (thoracic) spinal cord injury. Other disorders of interest to the U.S. Department of Veterans Affairs likely to benefit from development of a companion dog model include chronic pain, diabetes, cardiovascular disease, and senile dementia, including Alzheimer's disease. The utility of companion dogs may increase if other biomedical research areas wherein their use is scientifically valid

could be identified and if there is an infrastructure in place to facilitate the conduct of studies that use companion dogs.

Conclusion 4-2: A significant barrier to conducting clinical studies in companion dogs is a lack of administrative infrastructure to connect U.S. Department of Veterans Affairs (VA) investigators to the veterinary researchers who conduct such trials. The regulatory infrastructure to address ethical and legal issues for clinical trials in dogs is already established, but the mechanism for using these studies to supplement, complement, or accelerate collaborations with investigators who are interested in conducting human clinical trials does not exist. With validation of the utility and relevance of the naturally occurring canine disease or disorder for the study of the human equivalent disease or disorder, a network for developing a companion animal clinical trials registry could be created. The VA could move forward with supporting new collaborations to establish the relevance of dog studies to humans, both for conditions of likely future interest to the VA (e.g., posttraumatic stress disorder, natural infectious disease, Alzheimer's disease, and obesity) and for areas currently under study.

Conclusion 4-3: With respect to other animal models, rats and mice are the predominant species used for biomedical research in the fields of cardiovascular disease, spinal cord injury, cancer, and diabetes. For studies that cannot be performed in rodents (due to constraints of size, anatomy, or physiology), the pig has become the large animal translational model of choice. While pigs are not tractable for all areas, their potential uses are likely to expand in the near future as genetically modified strains become more widely available.

Conclusion 4-4: While the scientific and institutional animal care and use committee review processes at the U.S. Department of Veterans Affairs adhere to all relevant policies established by the U.S. government (as described in Chapters 2 and 5), compliance with these standards on its own may not be sufficient to ensure adequate identification and consideration of new approach methodologies (NAMs). Even in the case of protocols that still require the use of laboratory animals, researchers need to be encouraged to evaluate and incorporate NAMs where feasible.

Recommendation 4: Develop a strategic roadmap to create, track, and sustain internal efforts to incorporate new approach methodologies (NAMs) in U.S. Department of Veterans Affairs (VA) biomedical research.

The VA should establish a strategic roadmap and accompanying framework to promote the development and incorporation of NAMs to replace, reduce, or refine the use of dogs and all other laboratory animals in VA research. This framework should prioritize:

- **Modifying the protocol review processes (see Recommendation 3) to require and support robust consideration of NAMs, human clinical trials, companion dogs, and alternative animal models. The potential of these alternatives to contribute to the overall goals of the research, not just to replace laboratory dogs, should be considered.**
- **Incentivizing the use of NAMs. Examples of ways to do this include:**
  - **Developing and funding new VA grant opportunities to promote the development of NAMs that meet the unique needs of VA researchers, including the use of human tissues and organs, in vitro, in silico, and computational approaches.**

- **Funding for researchers and institutional animal care and use committees to undertake training in state-of-the-art, human-based methods to increase awareness and help establish confidence in these new approaches. Hands-on training and similar knowledge transfer opportunities will be particularly important and should be prioritized.**
- **Implementing compulsory funding to promote the evaluation and optimization of NAMs that address research objectives identified in studies that currently require the use of laboratory dogs (i.e., parallel funding requirements).**

Recommendation 5: Establish long-term external collaborations to optimize the use of companion dogs and humans in biomedical research.

The U.S. Department of Veterans Affairs should prioritize the development and continuation of external multi-disciplinary collaborations to develop, validate, and apply alternatives to the laboratory dog in biomedical research. This effort should result in the following:

- **Increased collaborations with external scientists and use of public–private partnerships to promote cross-sector communication and cooperation.**
- **The fostering of collaborations with researchers conducting clinical trials in companion dogs to identify or develop trials to benefit veterans and dogs.**
- **The encouragement of the use of human organs and tissues from human organ banks whenever possible.**

CARE AND WELFARE OF LABORATORY DOGS USED IN RESEARCH

Over the past 50 years, an impressive array of work has been done to understand the welfare of animals in a range of settings, from farms and laboratories to zoos, shelters, and the wild. To survey that work, even within the laboratory setting, is beyond the scope of this report, but four key conceptual advances from this period inform practice regarding the welfare of animals in human care today. These advances reflect developments in the field of animal welfare related to the following and are discussed in more detail in Chapter 5: animal sentience, the emergence of three distinct approaches to the nature and assessment of animal welfare, a consideration of positive and negative welfare states, and the recognition that welfare assessments incorporate both resource-based and animal-based considerations.

Conclusion 5-1: Animal welfare is multi-dimensional, reflecting the health, comfort, behavior, and emotions of animals in human care. There is a need for the U.S. Department of Veterans Affairs to combine aspects of each of the three major approaches to animal welfare, explicitly consider positive and negative welfare states, and measure animal-based welfare indicators in order to enhance the positive welfare of laboratory dogs.

Conclusion 5-2: While the Guide for the Care and Use of Laboratory Animals *and the Animal Welfare Act provide a foundation for the assessment of well-being, the U.S. Department of Veterans Affairs has an opportunity to incorporate current developments in animal welfare science into its animal care and use program.*

Conclusion 5-3: In the pursuit of enhancements to the care and use of laboratory dogs in U.S. Department of Veterans Affairs (VA) research, international regulations and guidelines may

be a useful resource for alternate approaches and recommendations. International guidelines and recommendations offer the VA programmatic leaders and institutional animal care and use committees expanded perspective on the contemporary literature and trends on laboratory dog care.

Some members of the committee conducted visits to two of the VA programs engaged in dog research in order to meet with personnel involved in oversight, care, and research on dogs and to observe the conditions and practices associated with this use. Materials obtained in advance of the National Academies site visit to the Richmond VA Medical Center indicated that practices relating to dog husbandry and care were sound and conformed with the applicable requirements. These materials included two letters of accreditation from AAALAC International, which strongly supported the VA's assertion of a high-quality program for the care and use of dogs. External review by the National Institutes of Health's Office of Laboratory Animal Welfare and upper management within the VA system also found provisions for the care of dogs at the Richmond VA Medical Center to be in compliance. Both the Richmond and St. Louis support facilities, such as surgeries and treatment rooms, were state of the art and well maintained.

Conclusion 5-4: Based on the information obtained during site visits and in materials submitted to the committee, including AAALAC International accreditation letters and the Office of Laboratory Animal Welfare report on assessment of the U.S. Department of Veterans Affairs (VA) canine research program, the committee concludes that the VA appeared to meet or exceed current regulatory requirements. Nonetheless, the committee observed several areas where the VA's animal program could be enhanced, and those enhancements are included in the recommendations below.

Recommendation 6: Enhance the welfare of laboratory dogs used for biomedical research.

The U.S. Department of Veterans Affairs (VA) should enhance the welfare of laboratory dogs used in biomedical research in their facilities in the following ways:

- **Submit to voluntary U.S. Department of Agriculture (USDA) inspections of dog facilities to increase transparency.**
  - **As a federal agency, the VA must meet all Animal Welfare Act requirements but is not subject to mandatory inspection by the USDA. Requesting voluntary USDA inspections of dog facilities would not only increase transparency (as summaries of results from USDA inspection reports are posted on a public website), but it would also provide another independent peer review of the VA's animal care practices and facility compliance from a neutral, independent third party.**
- **Modify dog enclosures and staffing to enhance opportunities for social interaction, exercise, and sensory stimulation.**
  - **Ensure staffing is to a level sufficient for all dogs to have 30–45 minutes outside of their primary enclosures 7 days per week.**
  - **Deploy a system of adjoining cages with barriers or transfer doors. When the facility is not fully occupied, this type of system would provide the dogs with more space, more behavioral choices, and more opportunities for exercise and could enable compatible dogs to have tactile contact.**
  - **To the extent compatible with the needs of studies, maximize the amount of time dogs are able to interact with humans or be let out of their primary enclosures.**

  - **When it is compatible with study goals and safe for the dogs and personnel, create an outdoor space for laboratory dogs to visit on a regularly scheduled basis. This would provide additional opportunity for exercise as well as olfactory, sensory, and visual stimulation; a variety of experiences; and time with humans.**
  - **Increase the amount of enrichment available to dogs, and continue to evaluate and incorporate new options for environmental enrichment, including olfactory enrichment, on a regularly scheduled basis.**
- **Address current experimental impediments to dog–dog interactions.**
  - **Given concerns about possible wounding or damage during the social housing of dogs wearing internal or external (implanted) devices, fund a pilot study at the end of an existing protocol to examine the risk of these outcomes.**
  - **Consider an alternative placement of implanted devices to decrease the likelihood of complications from socialization with other dogs.**
  - **Encourage the development and use of miniaturized devices that are less cumbersome for the animals and less likely to be damaged, especially if they can be implanted subcutaneously.**
- **Conduct enhanced assessments of laboratory dog welfare.**
  - **To move beyond simple observations of dogs' health, VA staff involved in the care and welfare of laboratory dogs should collaborate on continuous education and continuous improvement of measures that advance laboratory dog welfare.**
  - **VA veterinary and animal care staff, facilities personnel, members of the institutional animal care and use committee, and principal investigators should conduct formal, written assessments of animal welfare that reflect the state of the art in animal welfare assessment methods.**

1

Introduction

Vertebrate animals have occupied a central role in biomedical research since the early days of medicine, owing to their broad anatomical and physiological similarities to humans. Research on these animals is generally performed to satisfy one of several overarching goals: answer basic questions relevant to the understanding and treatment of human disease; develop and improve on medical interventions, such as surgical procedures; or evaluate the safety and efficacy of drugs, devices, or other therapeutic approaches before they can be tested in humans.

The dog (*Canis lupus familiaris*) was the first animal species to be domesticated, occurring at least 14,000 and perhaps as far back as 36,000 years ago. Over the millennia humans have bred dogs for multiple uses while selecting them for diverse traits, including sociability and obedience. The ubiquitous presence of dogs and their comfort with humans likely contributed to their use in biomedical research as early as the 1st century CE (Ovodov et al., 2011; Serpell, 2019), and it continues to do so to the present day. Although the precise numbers are unknown, dogs were arguably the primary vertebrate species in use from the late 19th to the mid-20th century (Kinter and DeGeorge, 2016).

In the latter part of the 20th century, mice and rats rapidly replaced dogs in the laboratory, and rodents remain the preferred subjects for the majority of contemporary studies (Franco, 2013). When a process cannot be effectively studied in rodents, then another species will be sought; commonly used species include rabbits, birds, guinea pigs, hamsters, pigs, sheep, non-human primates, cats, and dogs.

Many public and private institutions throughout the United States use animals for biomedical research purposes. The past half-century saw a steep decline in dog use reported by the U.S. Department of Agriculture (USDA), which tallies all dogs used for research in the United States, with 60 percent fewer dogs used in 2017 than in 1973[1] (USDA APHIS, 2020, n.d.). In the United States in 2017 (the most recent year with complete data when this analysis was initiated in mid-2019), 60,190 dogs were used for biomedical research and testing purposes, according to annual reports submitted to USDA as required by the Animal Welfare Act of 1966 (USDA, 2019). Of these,

[1] B. Juarez, USDA APHIS, personal communication, May 31, 2019.

22,933 dogs were reported by academic institutions and affiliated hospitals engaging in biomedical research and education; 34,875 by companies and private research organizations engaging in applied biomedical research and product development, including testing required by regulatory agencies; 832 by government organizations (including U.S. Department of Veterans Affairs [VA] research labs) conducting priority basic and applied research in support of their missions; and 1,550 by other, non-research groups. A more detailed discussion of USDA data and trends in dog use in research is presented in Appendix B.

The very character traits that make dogs attractive subjects for study also lead to concerns being raised about their use in biomedical research. Public vivisections of dogs elicited strong objections as early as the mid-1700s and inspired the anti-vivisection movement a century later (Patterson-Kane and Golab, 2014). Over a 140-year period leading to the present, a framework of laws and regulations was constructed to prevent the abuse of dogs used in research (see Chapter 2). These regulations also address the particular needs of dogs and other sensitive species (Serpell, 2019).

The VA, as part of its mission to care for America's veterans, supports and conducts a wide range of preclinical animal research to help advance scientific knowledge and the understanding of how diseases affect veterans (VA, n.d.). The ultimate goal of VA research is to restore or improve the health and well-being of veterans (Bever, 2019; Ramoni, 2019). All animal research performed at the VA is required to comply with the Animal Welfare Act of 1996 and its corresponding animal welfare regulations as well as U.S. Public Health Service policy, and such research is subject to approval and regular inspection by local institutional animal care and use committees (IACUCs). The regulations governing animal use and care at the VA are described in detail in Chapter 2 of this report.

Currently, most VA animal research studies use rodents. In fiscal year (FY) 2017, mice constituted 93 percent and rats 6 percent of all animals used at the VA. That year, a total of 119 dogs were used in VA biomedical research, constituting less than 0.05 percent of all animals used (VA, 2018). For FY 2018, VA animal research programs reported work with 83 dogs in 6 studies. For FY 2019, VA animal research programs reported research conducted with 55 dogs in 6 studies. A seventh study was in progress in FY 2019, but that work is a clinical trial with client-owned dogs (companion animals), which USDA reporting requirements exclude.

ORIGIN OF THIS STUDY

Although the use of animals in biomedical research is regulated by federal laws and is subject to institutional oversight to ensure the humane use and care of laboratory animals, this practice remains a contentious issue and a focus of intense public scrutiny, particularly in the case of dogs. In May 2017, reporting on an animal use protocol for an invasive study performed on dogs at the Greater Los Angeles VA facility found that the study had been mischaracterized as observational, leading some members of the U.S. Congress to question the transparency of VA-supported dog research and to further investigate dog research in the Greater Los Angeles VA facility (Garcia, 2017; Titus et al., 2017; Wire, 2016).

Also in May 2017, the VA Office of Research Oversight (ORO) reported on its investigation, Canine Research Studies and Associated Facility Oversight, carried out at the Hunter Holmes McGuire VA Medical Center (HHMGVAMC) in Richmond, Virginia, which had been undertaken to address seven allegations referred by the Office of Inspector General (VA ORO, 2017). The ORO substantiated three allegations relating to animal welfare, all of which had been previously self-identified and reported by VA personnel, with corrective actions taken by the local IACUC. The ORO made additional findings related to deficient recordkeeping, non-adherence to provisions of the facility's written program of veterinary care, and deviations from approved study procedures without prior IACUC approval. The HHMGVAMC took corrective actions to address these findings (VA, 2017). In the January 2019 Report on the Office of Laboratory Animal Welfare Site

Visits to the Veterans Affairs Medical Centers with Focus on Canine Care and Use in Research, the National Institutes of Health's Office of Laboratory Animal Welfare found that the HHMGVAMC "responded appropriately to the allegations of animal welfare concerns, recordkeeping and reporting inconsistencies" and "not only executed and implemented, but has consistently maintained, the appropriate corrective measures in response to the animal welfare concerns" (OLAW, 2019).

In December 2017 the VA required all research projects using dogs to undergo a secondary review by the Office of the Chief Veterinary Medical Officer after the initial review by the local IACUC, followed by additional reviews by senior Veterans Health Administration managers (VHA, 2018). Section 254 of the Consolidated Appropriations Act of 2018 (Public Law 115-141) included a stipulation that no new canine research could begin at the VA unless it was directly approved by the VA Secretary.[2]

In March 2018 the VA announced its intention to conduct an in-depth internal review of existing canine research projects (VA OPIA, 2018). The VA requested the National Academies of Sciences, Engineering, and Medicine (the National Academies) to convene an ad hoc committee that would conduct an independent assessment of whether dogs are or will continue to be necessary for any type of biomedical research directly related to the VA's mission. The charge to the committee is presented in Box 1-1. Note that non-human primates were excluded from the committee's consideration according to the Statement of Task.

BOX 1-1
Statement of Task

In response to a request from the U.S. Department of Veterans Affairs (VA), the National Academies of Sciences, Engineering, and Medicine will appoint an ad hoc committee to review the care and use of dogs in biomedical research funded by the VA or carried out in VA facilities, regardless of funding source, for the purpose of advancing science and the understanding of how diseases affect the health of veterans. Specifically, the committee will write a report to address the following:

1. Explore recent past, current, and anticipated research questions directly related to the VA's mission to determine if dogs [rather than non-rodent (excluding non-human primates) or rodent species or non-animal alternatives] are or will continue to be necessary for relevant basic and translational research. The committee will:
   a. Make a determination as to whether dogs are or will continue to be necessary for any type of biomedical research directly related to the VA's mission. If it is determined that they are necessary, describe the unique physiological and other characteristics of dogs that currently make it the necessary animal model for use in these types of research;
   b. Provide recommendations for any new or revised scientific parameters to guide how and when to use dogs for biomedical research rather than non-rodent (excluding non-human primates) or rodent species or non-animal alternatives; and
   c. Explore contemporary and anticipated future alternatives to the use of dogs in this research and determine how these could be part of a strategy to develop and/or use alternatives in support of the agency's mission.
2. Identify ethical considerations, regulatory requirements, and currently accepted standards for the care, use, and welfare of dogs in biomedical research, and make recommendations to enhance their well-being while achieving the research objectives.
3. Determine whether the VA's current review and oversight practices meet the standards, requirements, and recommendations identified above, and make a separate determination as to whether changes in VA practices are recommended.

[2] Consolidated Appropriations Act of 2018, H.R. 1625, 115th Cong., 2nd sess. (January 3, 2018). Available at https://www.congress.gov/115/bills/hr1625/BILLS-115hr1625enr.xml (accessed June 25, 2019).

RECENT LEGISLATION CONCERNING THE USE OF LABORATORY DOGS AT THE VA

On December 20, 2019, roughly 1 year after this committee was convened, additional constraints were placed on the use of dogs in biomedical research at the VA by the U.S. Congress, as detailed in Section 249 of the Further Consolidated Appropriations Act of 2020.[3] This regulation placed further restrictions on the ability of the Secretary of the VA to approve research using "canines, felines, or non-human primates," again limiting their use to conditions where "the scientific objectives of the research can only be met" by using these animals. The additional constraint was added that these animals could only be used where "such scientific objectives are directly related to an illness or injury that is combat-related." In addition, the legislation requires the VA Secretary to personally report all new research on canines, felines, or non-human primates to the U.S. Congress, with continued biannual reporting, and to submit a plan by December 31, 2020, for eliminating or reducing research on these species over the next 5 years.

The committee notes that the legislation raises three points of particular significance for its work:

1. While the committee was charged with considering the necessity of dog use "related to the VA's mission," the legislation restricts dog use to research that is "directly related to an illness or injury that is combat-related." The stated mission of the VA is "[t]o fulfill President Lincoln's promise 'To care for him who shall have borne the battle, and for his widow, and his orphan' by serving and honoring the men and women who are America's Veterans." The VA is charged with providing health care to veterans throughout their life span, and it engages in research that supports this charge, including research on cardiovascular disease, cancer, diabetes, and a range of other disorders that may no longer be eligible for study in dogs under this new legislation (see Chapter 3 for a discussion of ways in which dogs have historically been used at the VA). Likewise, the use of naturally infected military dogs to study infectious diseases that both dogs and soldiers contract during deployments overseas (also discussed in Chapter 3) may not be permissible under this stipulation.
2. The legislation does not distinguish between the use of laboratory dogs and the use of companion (pet) dogs in biomedical research. Unlike laboratory dogs, companion dogs have naturally occurring diseases or injuries, are volunteered by their owners, and stand to benefit from the results of the research. Public interest in and support for companion dog studies is generally high. The committee's recommendations rely heavily on the ability of the VA to move research from laboratory dogs to companion dogs where feasible as well as to find novel opportunities to engage in companion dog research where it would satisfy the VA's mission.
3. By restricting dog use to situations in which the scientific objective can only be met by using dogs, the legislation privileges dogs over other large mammals, such as pigs. In other words, VA researchers are required to use other animal species in all cases where the scientific objectives of the research can be met using other species as well as dogs. This is the case even when the particular scientific question could be addressed using fewer dogs than, for example, pigs, or would cause less harm to dogs than to pigs. Choosing to use pigs instead of dogs under these circumstances would constitute a potential contradiction of the Three Rs (reduce, refine, replace) that are used to guide ethical decisions in animal research and discussed in depth in Chapter 2.

[3] Further Consolidated Appropriations Act of 2020, H.R. 1865, 116th Cong., 1st sess. (January 3, 2019). Available at https://www.congress.gov/bill/116th-congress/house-bill/1865 (accessed January 24, 2020).

STUDY PROCESS

A Statement of Task guides each National Academies study and determines what kinds of expertise are needed on a committee. A committee writes a report to answer as rigorously as possible the questions posed in the Statement of Task.

Committee Formation

Members of the committee that conducted this study[4] were selected from among more than 100 persons nominated during the committee-formation phase of the study. Individuals appointed to the committee were chosen for their individual expertise and the relevance of their experience and knowledge to the Statement of Task (see Box 1-1), not their affiliation with any institution. All committee members volunteer their time to serve on a study. Areas of expertise represented on the committee included laboratory animal veterinary medicine, biomedical sciences, medicine, translational research, animal welfare science, animal welfare regulation, bioethics, and animal law. Biographies of the committee members are in Appendix C.

Public Input

As with all National Academies studies, members of the public were invited to provide oral or written statements and information to the committee, especially during the information-gathering phase of the study. The in-person meetings held in Washington, DC, in December 2018, February 2019, and March 2019 included time for members of the public to provide comments to the committee. Recordings of the public comment sessions are archived on the study's website.[5]

Written comments to the committee could be submitted at any point during the study process. Comments and information could be delivered to National Academies staff at committee meetings and via email. Members of the public could also submit comments or upload relevant documents to the study's website. More than 4,100 comments and documents were submitted to the committee, and the committee read all of them.

Some commenters told the committee in written statements or at its public meetings that the committee should make a decisive pronouncement endorsing the use of dogs in biomedical research as categorically beneficial. Others encouraged the committee to denounce the use of dogs in biomedical research. Comments were also submitted with literature on non-animal approaches, welfare, transparency in research, and the use of dogs in biomedical research.

[4] Every National Academies committee is provisional until the appointed members have had an opportunity to discuss as a group their points of view and any potential conflicts of interest related to the Statement of Task. They also determine whether the committee is missing expertise that may be necessary to answer questions in the Statement of Task. As part of their discussion, committee members consider comments submitted by the public about the committee's composition. The discussion takes place during the first in-person meeting of the committee. The committee is no longer provisional when it has determined that no one with an avoidable conflict of interest is serving on the committee and that its membership has the necessary expertise to address the Statement of Task.

The Committee on Assessment of the Use and Care of Dogs in Biomedical Research Funded by or Conducted at the U.S. Department of Veterans Affairs did not identify any conflicts of interest among its members. However, in light of comments received from the public before its first meeting and because of two resignations, two new members with experience as American College of Laboratory Animal Medicine board-certified veterinarians and with service on an institutional animal care and use committee, one member with animal research ethics and law experience, and two members with biomedical research experience were added to the committee.

For more information about the National Academies study process, including definitions and procedures related to points of view and conflicts of interest, visit http://www.nationalacademies.org/studyprocess (accessed January 8, 2020).

[5] The study website includes recordings of public sessions; see https://www.nationalacademies.org/our-work/assessment-of-the-care-and-use-of-dogs-in-biomedical-research-funded-by-or-conducted-at-the-us-department-of-veterans-affairs (accessed June 16, 2020).

To address the Statement of Task, the committee drew on information presented during public meetings, webinars, and the workshop. The committee typically requested additional data or documentation from invited speakers following their presentations. It also reviewed statements and articles that were submitted or referred to by speakers or members of the public, and it thoroughly consulted the relevant peer-reviewed scientific literature.

Committee Deliberations and Information-Gathering Activities

To address the study charge, the committee deliberated from December 2018 to December 2019, holding six meetings (five meetings in Washington, DC, and one in Woods Hole, Massachusetts), including one 2-day public workshop (March 27–28, 2019, in Washington, DC), public comment sessions (during the open sessions of four committee meetings), and two webinars (May 7, 2019, and May 28, 2019). Agendas for the committee meeting open sessions, 2-day workshop, and webinars are included in Appendix A. Subgroups of the committee with expertise in laboratory animal care and welfare also visited facilities conducting VA-funded research in Richmond, Virginia (August 20, 2019), and St. Louis, Missouri (November 14, 2019). Throughout the study, the committee also received input from interested stakeholders and the public via the study website. All submitted comments and documents were added to the study's public access file, which is available on request from the National Academies' Public Access Records Office. Requests can be directed to PARO@nas.edu.

Information from the VA

The VA submitted several documents to the committee during the course of the study, including information surrounding 14 biomedical research protocols. For each of the 14 protocols, the VA included the animal component of research protocol form, a secondary review form, and a summary of the literature search done by the Office of the Chief Veterinary Medical Officer. The VA also submitted summaries of 30 past research projects involving dogs as well as representative publications from 28 of these projects ranging in date from 1960 to 2018. To evaluate the impact of this research, the committee reviewed the scientific literature in the decade 2009–2018, with the goal of understanding the contribution of past VA research involving dogs to clinical questions related to the VA's mission. A list of all the documents provided by the VA can be found in Appendix A of this report.

Adding to the information in the documents received from the VA, several public sessions allowed the committee to delve further into the context for the study and the Statement of Task as provided by the VA. During open sessions, VA representatives discussed the relevance of dog research to the VA's mission and current procedures for evaluating and monitoring dog use and care (December 9, 2018); addressed follow-up questions from the committee in writing and in person (February 14, 2019); and described the procedure for internal review and funding of research grants (March 28, 2019). Video recordings and slides from all VA presentations are publicly available from the study website.[6]

Report Review Process

The concluding phase of a National Academies report is the review process. When a draft report is complete, it is submitted to the National Academies' Report Review Committee (RRC).

[6] The study website includes recordings of public sessions; see https://www.nationalacademies.org/our-work/assessment-of-the-care-and-use-of-dogs-in-biomedical-research-funded-by-or-conducted-at-the-us-department-of-veterans-affairs (accessed June 16, 2020).

The RRC recruits a diverse and critical group of reviewers who have expertise complementary to that of the committee to ensure that critical gaps and misinformation are identified. The reviewers are anonymous to the committee during the review process, and their comments remain anonymous after the report is published (see the Reviewers section on p. vii). Reviewers are asked to assess how well a report addresses a study's Statement of Task. The committee must respond to, but need not agree with, reviewers' comments in a detailed "response to review" that is examined by one or two independent report review "monitors" responsible for ensuring that the report review criteria have been satisfied. When the RRC decides that the committee has adequately and appropriately addressed the reviewers' comments, the report is ready to be released to the public and to the sponsor.

THE COMMITTEE'S INTERPRETATION OF ITS TASK

In this study, the committee addressed the care and use of laboratory-housed dogs and not of privately-owned companion dogs enrolled in clinical trials. In accordance with the committee's charge, recommendations contained in this report focus on the committee's determination as to whether laboratory dogs are or will continue to be necessary for biomedical research conducted at or funded by the VA.

As one component of its effort to understand the procedures governing animal care and use at the VA and the scientific justification for undertaking research in dogs, the committee reviewed elements of research protocols provided by the VA. With a few exceptions as noted in the text, this report does not critique individual protocols.

The committee supplemented information from the VA panels with a review of the current scientific literature regarding clinical questions related to the VA's mission that have traditionally used dogs, taking into account both animal and non-animal alternatives to laboratory dog use (see the literature search parameters in Appendix A). In applying expert opinion to the issues raised in the study charge, the committee was guided by scientific, social, regulatory, and ethical considerations.

TERMINOLOGY USED IN THIS REPORT

The committee was faced with competing terminologies for describing the analytical process that weighs the risk of harm to laboratory dogs against the potential benefit of the research to humans—specifically, it had to choose between the terms "harm–benefit analysis" and "risk–benefit analysis." The semantics remain a topic of continuing debate among many stakeholders; Box 1-2 offers a brief background and explanation of the committee's decision. Ultimately, the committee decided to use "harm–benefit analysis" in this report in order to be consistent with the terminology used in VA documents (see Box 1-2). It was beyond the scope of this committee's work to make a determination regarding the scientific, ethical, and regulatory merit of the competing terminologies, and the use of "harm–benefit analysis" in this report should not be construed as a recommendation of this committee, the Institute for Laboratory Animal Research, or the National Academies.

ORGANIZATION OF THE REPORT

To produce this report, the committee identified scientific, social, legal, and ethical considerations; regulatory requirements; and currently accepted standards guiding the use, care, and welfare of laboratory dogs at the VA (Chapter 2). After surveying past and current dog use in biomedical research in fields relevant to the VA mission and reviewing current and recent dog protocols at the VA, the committee established criteria to guide the decisions of *whether* and *how* to use laboratory dogs in biomedical research at the VA (Chapter 3). The committee then

BOX 1-2
Terminology Related to Weighing the Risk of Harm Against Potential Benefit

The Animal Welfare Act and Regulations stipulate that "[p]rocedures involving animals will avoid or minimize discomfort, distress, and pain to the animals" and require appropriate use of anesthesia, analgesia, or sedation, in consultation with the attending veterinarian or his or her designee (USDA, 2019; specifically Subpart C—Research Facilities, 2.31 Institutional Animal Care and Use Committee [IACUC], pp. 56–60). The standard for implementing this rule is the Institute for Laboratory Animal Research's *Guide for the Care and Use of Laboratory Animals* (the *Guide*) (NRC, 2011), which assigns responsibility to the IACUC, as follows: "Certain animal use protocols include procedures or approaches that require special consideration during the IACUC review process due to their potential for unrelieved pain or distress or other animal welfare concerns…. For these and other areas the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns."

To describe this process of weighing study objectives against animal welfare concerns, some U.S.-based organizations, which must adhere to the *Guide*, employ the term "risk assessment" or "risk–benefit analysis." Some U.S. organizations use the terminology "harm–benefit." The term "harm–benefit analysis" is more common in Europe.[1] These terms carry distinct connotations, and choosing which to employ can be fraught. Use of "harm–benefit analysis" rather than "risk–benefit analysis" or alternative terminology may seem to imply that injury will necessarily be inflicted, and concern has been raised that use of the word "harm" may bias the public against animal research (Grimm, 2015, 2017; Kinter and Johnson, 2015; Simmonds, 2018). In fact, one early and influential definition of "harm" includes any impingement on the five freedoms, described as freedom from hunger or thirst; freedom from discomfort; freedom from pain, injury, or disease; freedom to express normal behavior; and freedom from fear and distress (Beauchamp and DeGrazia, 2019; Brambell, 1965; Mellor and Reid, 1994; Webster, 2005). According to this definition, housing animals for research purposes itself could be interpreted as causing harm (Davies, 2017). It was recently proposed that humane euthanasia be included among the list of harms (DeGrazia, 2019); extending the definition is a matter of continued debate. (Approaches to assessing welfare are discussed in detail in Chapter 5.)

In addressing the need to weigh the impact of laboratory dog research on the dog subject against its likely benefit to veterans, the committee elected to use the term "harm–benefit analysis." This decision was driven by the U.S. Department of Veterans Affairs, which, in its Animal Component of Research Protocols documents, requires every researcher proposing to do an animal study to satisfy the need for a "harm–benefit analysis" with a description of "how these benefits [to the health of people, other animals, or society] outweigh the pain or distress that may be caused in the animals."

[1] The European usage likely originates in Article 38 of EU Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes," which requires that evaluations of research projects involving animals include harm–benefit analyses. Available at http://data.europa.eu/eli/dir/2010/63/oj (accessed January 28, 2020).

reviewed current and likely future alternatives to the use of laboratory dogs in fields relevant to the VA mission (Chapter 4). Finally, the committee evaluated current review and oversight practices governing laboratory dog care at the VA and made recommendations to enhance the care and welfare of dogs in VA facilities (Chapter 5).

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2

Legal, Social, and Ethical Considerations

This chapter begins with a brief history of the use of dogs in research and of efforts to obtain legal protections for dogs as research subjects. This is followed by synopses of the regulatory requirements that govern the use of dogs at the U.S. Department of Veterans Affairs (VA) and of the currently accepted standards for the use, care, and welfare of dogs in biomedical research. Reflections on social and ethical considerations regarding the use of laboratory dogs in biomedical research are followed by the committee's conclusions with respect to the role of societal preferences in considering the use of dogs for biomedical research at the VA.

HISTORICAL OVERVIEW OF RESEARCH USING DOGS

From antiquity through the 18th century, experimentation on living creatures was used to obtain basic knowledge about anatomy and physiology as well as to develop surgical procedures. Humans—typically slaves, condemned criminals, the dead, or the investigators themselves—were the most frequently used species for these investigations. Non-human animals were also used at least as far back as Aristotle (Altman, 1998; Appleman, 2020; Kinter and DeGeorge, 2016; Lairmore and Ilkiw, 2015; Moore, 2006; Weisse, 2012; West, 2015).

Until the mid-20th century, investigations continued to be conducted in humans, including felons, the disabled and infirm, and orphans (Appleman, 2020). By the end of this period, incidents of unethical human research led to growing concern regarding the use of humans without their explicit and informed consent (Dankar et al., 2019). The Nuremberg Code, a product of the 1946–1947 trial of Nazi doctors for horrific medical war crimes, established 10 ethical principles for conducting legitimate (human) clinical research. These principles emphasize the need for informed consent and include a prerequisite for conducting animal research prior to human experimentation (Weindling, 2001). The Nuremberg Code prompted the World Medical Association to create the Declaration of Helsinki, which articulates similar principles. Since its adoption in 1964, the Declaration of Helsinki has undergone several revisions (WMA, 2018). By largely recapitulating and extending the themes raised by the original Declaration of Helsinki, the United States developed the Code

of Federal Regulations Title 45 Part 46, which was issued by the U.S. Department of Health and Human Services to lay out requirements for the ethical treatment of human subjects.[1]

Notwithstanding ethical and legal imperatives to conduct animal studies prior to engaging in human subjects research, most of the increase in animal use in the early to mid-20th century can be traced to developments in pharmacology, medicinal chemistry, and toxicology (Kinter and DeGeorge, 2016). With the explosion in pharmaceutical research and large-scale product development in the late 19th and early 20th centuries came periodic public health disasters, such as the death of 13 children from a commercial diphtheria antitoxin contaminated with tetanus in 1901, which led to adoption of the U.S. Biologics Control Act of 1902 (DeHovitz, 2014). This was followed by the Elixir Sulfonilamide disaster in 1937, in which (absent a requirement for toxicity testing) 105 people died of kidney failure after receiving an antibiotic that had been formulated in a toxic solvent, and thalidomide in the 1950s (Caron et al., 2016; Wax, 1995). In response to these episodes, the U.S. Congress passed the 1938 U.S. Food, Drug, and Cosmetic (FD&C) Act, followed by the 1962 Kefauver Harris Amendment, which required sponsors to demonstrate that their regulated products be both effective and safe before being granted marketing approval (Kinter and DeGeorge, 2016). The 1938 FD&C Act and the 1962 Kefauver Harris Amendment (also known as Drug Efficacy Amendment) birthed a new sub-discipline, regulatory science, for carrying out efficacy and safety evaluations of new regulated products (FDA, 2017). Subsequent regulatory guidance introduced new standard practices, including the requirement for testing in multiple (rodent and non-rodent) species (Kinter and DeGeorge, 2016). These regulations helped establish confidence among regulators, patients, and the public that new regulated products would be effective and safe, although at the cost of increased animal testing (Kinter and DeGeorge, 2016).

Dogs as Research Subjects

There is evidence that dogs were the first animal to be domesticated, having been derived from the grey wolf at least 14,200 and perhaps as long as 36,000 years ago (Ovodov et al., 2011). Unlike other domesticated species such as pigs and cows, which were primarily selected for traits related to food production, the ancestors of dogs were initially selected for their behaviors (Serpell and Duffy, 2014). The development of the human–dog relationship is often described as "co-evolution," emphasizing a theory of human–wolf cooperation that finds support in a recent study of modern-day indigenous populations (Pierotti and Fogg, 2017).

Genetic evidence indicates that the integration of dogs into human society amounted to a form of natural selection (independent of later selections aimed at generating specific breeds). That is, as a result of an increased role in human society, domestic dogs have evolved genetically to display behaviors—such as hypersociability—that make them well-suited to partnerships with humans (Cagan and Blass, 2016; Hecht et al., 2019; vonHoldt et al., 2017). Some of these same traits have, on the one hand, made dogs an attractive species for laboratory research, while on the other hand engendered strong public reactions to their use as laboratory animals.

While records are incomplete, it is probable that dogs were used as research subjects from antiquity because they, like people, were available and tractable. It is known that animals were used by 16th-century scientists to elucidate the structure and function of body systems, with dogs preferred for studies of anatomy, surgery, circulation, and cardiac, pulmonary, and renal functions. Dogs and other animals were also used as surrogates for humans when the use of cadavers was banned (Kinter and DeGeorge, 2016; Lairmore and Ilkiw, 2015; Moore, 2006).

[1] CFR Title 45, Part 46: Protection of human subjects. Available at https://www.govinfo.gov/app/collection/cfr (accessed October 7, 2019).

Beginning in the 19th century, aided by the growth of technologies to measure experimental endpoints, animal experimentation became increasingly sophisticated, addressing questions of organ morphology, physiology, and pathophysiology (Kinter and DeGeorge, 2016). Photographic images of a bulldog standing for the first electrocardiogram recordings helped ensconce dogs as the favored non-human model for cardiovascular research (Burchell, 1987; Nishida et al., 2010). Anatomical and physiological similarities between dog and human cardiovascular systems, elucidated over a century and a half, bolstered that perception (e.g., Hayashi et al., 1995).

Most current dog use in the United States is to fulfill safety testing requirements for regulated pharmaceutical and medical devices (Kinter and DeGeorge, 2016). Some of the documents that provide guidance to investigators, including the U.S. Food and Drug Administration (FDA) Redbook 2000, specifically reference dogs as a preferred non-rodent species (FDA, 2007). Addressing the two-species requirement for animal toxicity studies, the Redbook comments that the non-rodent species is "usually dogs" or, in screening for neurotoxicity, "preferably dogs" (FDA, 2007).

With the rise in new cellular and molecular disciplines and gene-manipulation technologies that favor the use of rodents, the late 20th century saw a precipitous decline in dog use in the United States, as described in Chapter 3 and Appendix B. The precise nature of this decline is discussed in greater detail in Chapter 3, which addresses the current use of dogs for biomedical research. While most laboratory dog use is carried out to satisfy regulatory requirements related to product development by private industry, investigators in a limited number of biomedical fields continue to perform research using laboratory dogs, based on familiarity, equipment, training and experience, access to historical data, biological factors that cannot be modeled effectively in other systems, or a combination of these reasons, as will be described in Chapter 3.

LEGAL CONTEXT FOR USING LABORATORY DOGS IN BIOMEDICAL RESEARCH

In the United States, the 1965 exposure of abuses by "Class B" unregulated dog dealers was a flashpoint in the evolution of attitudes toward the treatment of dogs and other animals, contributing, in part, to the passage of the Animal Welfare Act of 1966 (AWA) and subsequent amendments (Adams and Larson, 2016; Engber, 2009). The AWA established the basis for regulation of animal research involving selected warm-blooded animal species in the United States.[2]

For the past half-century, a set of principles known as the Three Rs (replace, reduce, refine) has guided the ethical use of animals in research (Russell and Burch, 1959; Tannenbaum and Bennett, 2015), and these principles are endorsed or incorporated into various regulations, policies, and laws, including the *Guide for the Care and Use of Laboratory Animals* (the *Guide*) (Griffin and Locke, 2016). The Three Rs require researchers to employ methods that replace the use of animals wherever possible, reduce the number of animals used, and refine procedures to minimize pain, suffering, distress, or lasting harm and improve animal welfare (Russell and Burch, 1959). Multiple U.S. and international organizations exist to advance the Three Rs and to enable scientific practice to draw ever closer to an ideal expression of these principles (Anestidou et al., 2017; EURL-ECVAM, 2019; ILAR, 2019; NC3Rs, 2019). Some ethicists and animal welfare scientists have proposed revisions to the Three Rs to reflect society's evolving understanding of animal welfare (see Beauchamp and DeGrazia, 2019; Brønstad and Berg, 2011; DeGrazia, 2019; Kirk, 2018; Mellor, 2016).

[2] U.S. Code Title 7, Chapter 54: Transportation, sale, and handling of certain animals. "Animal Welfare Act." Available at https://www.nal.usda.gov/awic/animal-welfare-act (accessed December 30, 2019).

Requirements for the Use of Dogs in VA Biomedical Research

Like most institutions that conduct animal research in the United States, the VA is required by law to comply with federal regulations under the AWA. Additionally, VA policy requires compliance with the U.S. Public Health Service (PHS) Policy, which includes adherence to American Veterinary Medical Association (AVMA) euthanasia guidelines and the provisions of the *Guide*. The VA recognizes that for each of these, the authority to interpret the requirements rests with the entity that published and oversees compliance with it. The VA also requires all VA animal research programs to maintain full accreditation by AAALAC International and to comply with internal VA policies and reporting requirements.

The Animal Welfare Act and Regulations

The U.S. Department of Agriculture (USDA) enforces federal regulations governing the care and use of dogs in biomedical research in accordance with the AWA, which aims "to insure that animals intended for use in research facilities or for exhibition purposes or for use as pets are provided humane care and treatment," and with the corresponding Animal Welfare Regulations (AWR).[3] Under the AWA/AWR, all institutions using dogs for research must register with the Secretary of Agriculture and procure dogs in accordance with the regulations. Minimum standards are established for the transportation, handling, housing, feeding, watering, sanitation, ventilation, and veterinary care of all research animals covered by the AWR, with additional requirements for exercising dogs. For animals involved in experimental procedures, standards include requirements to minimize pain and distress and to consider alternatives to procedures that may cause pain or distress.

Institutions registered under the AWA/AWR are required to keep records which are examined during annual unannounced inspections by the USDA veterinary medical officer. The VA's animal research programs are generally not visited by USDA inspectors but are still required to file a USDA annual report of animal usage and pain category for species regulated by the USDA (including dogs).

Institutional Animal Care and Use Committee

Each research institution must establish an oversight and ethical review committee, the institutional animal care and use committee (IACUC), which is required to inspect the institution's "program for humane care and use of animals" and "animal facilities, including animal study areas" at least semi-annually. For any activity involving animals, the IACUC must review those components relating to animal care and use for compliance with the AWA. Research with animals, including dogs, may be conducted only after the IACUC (1) reviews the protocol describing what is to be done, and why, and how discomfort, pain, and distress to the animals will be avoided or minimized; (2) determines that the protocol is in compliance with AWA/AWR, PHS Policy, and AAALAC International and VA requirements; and (3) grants approval for it to proceed. The IACUC must be made up of no fewer than five voting members, appointed by the chief executive officer of the institution, which for VA stations is the local medical center director. The IACUC is required to include at least "one Doctor of Veterinary Medicine with training or experience in laboratory animal science and medicine, who has program authority and responsibility for activities involving animals at the institution,"[4] one practicing scientist experienced in research with animals, one

[3] CFR Title 9, Subchapter A: Animal welfare. "Animal Welfare Regulations." Available at https://www.nal.usda.gov/awic/animal-welfare-act (accessed December 30, 2019).

[4] CFR Title 9, Chapter 1, Subchapter A, § 2.31(b)(3)(i) and PHS Policy, par IV.A.3.b(1).

member whose concerns are primarily nonscientific, and one member who is otherwise unaffiliated with the research institution.

Work on any research with animals may begin only after the IACUC determines that the proposed activities are in accordance with regulatory requirements. Those requirements focus on "avoid[ing] or minimize[ing] discomfort, distress, and pain to the animals, consistent with sound research design,"[5] which implies a responsibility to weigh study objectives against animal welfare concerns. The standard VA animal protocol form, the animal component of research protocol, approaches this responsibility in several ways, including requiring documentation of (1) harm–benefit analysis, (2) justification for the number of animals requested, (3) justification for the species requested, (4) endpoint criteria, and (5) a database search for appropriate alternatives, in accordance with the tenets of the Three Rs, to replace animals, reduce the number of animals used, or refine procedures to better minimize pain or distress. The IACUC has the authority to withhold approval from any research proposal and to stop work on any project it determines to have failed to meet proper standards, and the IACUC is required to report noncompliance to the VA's facility director and the associate chief of staff for research and development. The IACUC does not itself evaluate the scientific merit of a study but rather relies on the judgment of the committees of scientific subject-matter experts that carry out scientific review of the research proposal.

The Office of the Chief Veterinary Medical Officer provides in-person animal research and IACUC training for VA personnel once or twice per year at major national meetings and also in customized station-specific training workshops for IACUC members conducted at individual stations as needed. IACUC training exercises are developed about four times per year to help local IACUCs maintain expertise and stay abreast of emerging issues (NRC, 2011).

The U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals

PHS Policy, administered by the National Institutes of Health's (NIH's) Office of Laboratory Animal Welfare (OLAW), is specifically designed to apply to the care and use of animals involved in research, research training, and biological testing activities conducted or supported by PHS (PHS Policy, par. I), which includes institutions receiving research funds from NIH, FDA, or the Centers for Disease Control and Prevention. The VA has a longstanding arrangement for OLAW to oversee all VA research with animals, so any VA research with animals must be covered by a PHS animal welfare assurance (statement of compliance) approved by OLAW, regardless of whether the activities use PHS funds. OLAW recently began overseeing animal activities funded by the National Science Foundation and the National Aeronautics and Space Administration as well. Compliance with PHS Policy requires adherence to the recommendations in the *Guide* (NRC, 2011) and relies on the guidance about the PHS Policy provided in the Frequently Asked Questions guidance section of the OLAW website (OLAW, 2019).

The *Guide* is intended to assist institutions and investigators in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate in accord with scientific, humane, and ethical principles. The *Guide* highlights a commitment to the Three Rs and provides detailed recommendations regarding the composition and responsibilities of the IACUC, including guidelines for protocol review and post-approval monitoring (NRC, 2011). The *Guide* also includes detailed recommendations for housing and environment (including the importance of social housing), veterinary care, and the physical plant. Its recommendations are presented as "must," "should," or "may," indicating practices deemed mandatory, strongly recommended, or suggested for consideration. Any deviation in practice from a "must" statement requires written justification and inclusion in a semi-annual report to the institutional official, typically the medical facility

[5] PHS Policy IV.C.1.a and CFR Title 9, Chapter 1, Subchapter A, § 2.31(d)(1)(i).

director. The *Guide* stipulates that primary oversight responsibilities for an animal care and use program rest with the institutional official, the attending veterinarian, and the IACUC.

The American Veterinary Medical Association's Guidelines for the Euthanasia of Animals

AVMA's *Guidelines for the Euthanasia of Animals* (AVMA, 2013) provides veterinarians with guidance for relieving the pain and suffering of animals that are to be euthanized, consistent with the obligations of the Veterinarian's Oath. Methods for measuring consciousness, pain, stress, and distress are discussed, as are principles governing the administration of inhaled, non-inhaled, and physical agents. Intravenous injection of a barbituric acid derivative is the preferred method of euthanasia for dogs. Euthanasia for dogs in laboratory settings must be approved by the IACUC.

AAALAC International

AAALAC International is an internationally recognized accreditation organization that evaluates institutions that voluntarily apply for accreditation. VA policy requires all facilities where VA animal research is conducted to be in programs that maintain full accreditation. AAALAC International performs site visits of every accredited program once every 3 years, using a peer-review process and site visits by teams selected for their professional expertise. Each VA program that conducts dog research receives an additional AAALAC International interim site visit in between the usual triennial visits. AAALAC International uses the *Guide* (NRC, 2011) as a primary standard for accreditation.

Internal VA Policy Documents

In addition to the external requirements listed above, VA animal research programs are required to comply with the following internal VA policy documents:

- Veterans Health Administration (VHA) handbook 1200.07, *Use of Animals in Research* (VHA, 2016), sets forth principles and procedures that govern research, testing, and teaching activities involving laboratory animals at the VA, including detailed requirements for the composition and responsibilities of the IACUC. Training is required for all individuals involved in animal research and husbandry; the American Association for Laboratory Animal Science provides web-based training tools for VA husbandry staff (AALAS, 2020). The current forms required by the VA for animal research programs and detailed instructions for their use are available online.[6]
- The animal component of research protocol is the form required for protocols submitted for IACUC review, for projects that are to be supported by VA funding. The forms for VA IACUC semi-annual evaluations of the animal research program and facilities provide checklists as well as tables for documenting the significance of deficiencies noted and how the IACUC determines they are to be addressed as well as a framework for summarizing the findings as a meaningful guide for improving the program and moving forward.
- VHA handbook 1058.01, *Research Compliance Reporting Requirements* (VHA, 2015), sets forth the requirements for reporting non-compliance events in VA research to research review committees, facility officials, and the Office of Research Oversight (ORO). According to the handbook,

[6] See https://www.research.va.gov/programs/animal_research/documents.cfm (accessed August 20, 2020).

> VA personnel … must ensure written notification of the IACUC within 5 business days after becoming aware of any apparent unanticipated death(s) of animals used for research, including deaths due to physical plant deficiencies, engineering failures, worker errors, test article toxicity, anesthetic or surgical complications, and other mishaps…. The IACUC must notify the VA facility director and the ACOS/R&D [associate chief of staff for research and development] within 5 business days after reaching a determination that a reportable incident has occurred…. The VA facility director must report the incident to ORO within 5 business days after receiving the IACUC's notification.

STANDARDS FOR THE USE AND WELFARE OF DOGS IN BIOMEDICAL RESEARCH

AWR (Part 3, Subpart A) set specific standards for dogs used in research with regard to identification, transportation, emergency planning, housing, and husbandry.[7] Dogs must be individually identified and provided with safe containment, temperature control, and adequate food and water during transportation. Dog enclosures must be of an adequate size (based on dog size) and constructed of sanitizable materials that are regularly cleaned and safe for the animals. Ventilation, temperature, and humidity must fall within prescribed parameters, and lighting must be diurnal and not excessive. Though laboratory dogs are rarely housed outdoors, there is a separate set of standards that must be met to provide for their comfort and safety in this circumstance. Health and husbandry standards stipulate the need for the compatibility of dogs housed together, the regular exercise of dogs housed in individual enclosures, daily feeding and twice-daily watering, daily cleaning of primary enclosures, biweekly sanitization of used primary enclosures and food and water receptacles, housekeeping, and pest control. Medical care must be provided and overseen by an attending veterinarian. Employees must be adequately supervised and trained to carry out the required level of animal husbandry and care.

The *Guide* (NRC, 2011) provides detailed recommendations regarding the dogs' environment, adding noise and vibration to the factors mentioned above along with a consideration of their socialization needs. Recommendations for medical care place a strong emphasis on the need to minimize pain and distress. Unlike the AWA/AWR, most recommendations in the *Guide* fall in the categories of "should" or "may" rather than "must."

Conclusion 2-1: Based on the documentation provided by the U.S. Department of Veterans Affairs (VA) and other organizations (e.g., confirmation of AAALAC International accreditation) as well as site visits, the committee concludes that the VA's biomedical research programs involving laboratory dogs appear to adhere to all relevant policies surrounding animal research.

SOCIAL AND ETHICAL CONSIDERATIONS

The committee was convened specifically to assess the use of dogs within the United States, and it is reasonable to ask why public and political pressure is being placed on the VA to curtail dog research in particular. The perceived mistreatment of dogs elicits an intense emotional reaction that resonates in the public sphere. As noted previously, it was this dynamic that led to passage of the AWA. The U.S. Congress passed the AWA in response to public pressure triggered in part by a *Life*

[7] CFR Title 9, Subchapter A: Animal welfare. "Animal Welfare Regulations." Available at https://www.nal.usda.gov/awic/animal-welfare-act (accessed December 30, 2019).

magazine article exposing the neglect of dogs housed by unlicensed animal dealers—an article that stimulated more letters to *Life* magazine than any story on Vietnam or civil rights (Stevens, 1990).

The committee did not investigate the basis for valuing dogs differently from the many other species that share humans' lives, habitats, and research activities. Pigs, cats, birds, horses, livestock, and an array of other species have their proponents. The idea that dogs should receive preferential treatment over other domesticated species is not supported by the current understanding of ethics as it applies to animals (DeGrazia, 1996). In other words, from an ethical standpoint—discussed in greater detail later in this chapter—dogs are not intrinsically more valuable than other large mammals.

ETHICAL CONSIDERATIONS ON THE USE OF DOGS IN VA RESEARCH

Dogs are valued by members of the public, including many veterans, and they tend to receive privileged treatment relative to other non-human species. This is evidenced in the amount that Americans spend on companion animals as well as by some of the evolutionary evidence suggesting that dogs have been bred and that perhaps in ancient times *Homo sapiens* selected dogs for preferential treatment.

However, the prevalence and intensity of such preferences (and their biological basis) has less than obvious implications for the ethics of using laboratory dogs in research. On the one hand, it is tempting to honor such dominant societal preferences by privileging dogs and enacting unusually restrictive policies for conducting research using them. On the other hand, cultural preferences sometimes lead society astray in terms of ethical decision making. One need not look far back in U.S. history to find moral mistakes where dominant social preferences supported policies that privileged members of certain groups. A more developed model of the ethics of using laboratory dogs in VA research requires an analysis of how the interests of veterans, laboratory animals, members of society, and others are implicated by conducting such research and what duties the VA has to members of those various groups.

Research involving laboratory dogs implicates the interests of laboratory dogs; knowledge users like patients, physicians, and veterinarians; non-canine laboratory animals that might be substituted for or replaced by laboratory dogs; working, service, military, and companion dogs that may derive benefit from research using laboratory dogs; members of society who place a high value on dogs; and individuals who depend on the institution under whose auspices the research is conducted—in this case, the VA. An ethical framework for biomedical research with laboratory dogs should explain how the interests of each articulate with each other, which interests should drive decisions and policy, and how interests and preferences might be accommodated or respected without violating obligations.

There is widespread consensus within the field of bioethics that many non-human animals have moral status. Though the views of different commentators often originate from different ethical theories, they generally converge on the position that members of any non-human animal species that has sentience deserve protection and respect (DeGrazia, 1996; Regan, 1983; Singer, 1975). Another way this is commonly articulated is that the suffering endured by a member of one species does not, by virtue of its species membership alone, deserve greater or lesser regard than the equivalent amount of suffering in another species.

Dogs clearly qualify as having sentience and various mental states that entitle them to respect and protections in biomedical research. For example, they have interests in their own welfare. They feel pain, anxiety, fear, and depression, and they have preferences for being treated in certain ways. Dogs also have the capacity to flourish in a life. In these respects, dogs are not different from other sentient species such as human beings, non-human primates, cats, pigs, and mice. Where species vary is in their particular needs—that is, what they need to flourish and avoid suffering. For

example, whereas dogs might need social stimulation with other dogs or human beings in order to thrive, pigs might require a different type of social stimulation or none at all.

Some ethicists and anti-vivisectionists would advocate a ban on any invasive research involving members of sentient non-human animal species. However, the present report is working within assumptions grounding the Statement of Task, one of which is that animal research can be conducted ethically. To that end, policy and regulations have established several ethical conditions for conducting animal research. These include the following:

- Any experiment should minimize compromises to the welfare of a non-human animal by using the smallest number of animals possible, by using the least invasive and burdensome approaches possible (including using companion dogs instead of laboratory-housed dogs where feasible), or by substituting species that are expected to have their welfare less compromised (or using in vitro or in silico methods);
- All animals used in research must be treated humanely. Specifically, non-human animals must receive competent veterinary care, must be given proper analgesia and must be euthanized if in extreme duress, and must be housed under conditions that are suitable and enriched for that species;
- Any experiment involving non-human animals should be conducted according to high scientific standards;
- The burdens of any experiment involving non-human animals must be justified by the value of the knowledge acquired from the experiment; and
- Judgments regarding the fulfillment of each of the requirements above should be independently and expertly refereed.

In recent years, various commentators have sought to extend these standards—for example, by requiring that non-human animals used in research should have "lives worth living" (DeGrazia and Sebo, 2015). Of course, having standards does not mean that the application of those standards is done easily or without controversy. For example, quantifying the value of the knowledge to be attained from a study is contentious. For instance, must it be directly translatable to human (or veterinary) use, or is an expansion of a body of knowledge sufficient? Most of these questions lie outside the scope of this committee's task. Dogs, nonetheless, have unique needs and interests in being treated humanely in the context of research. Chapter 5 contains a detailed discussion of the care and welfare of laboratory dogs.

Substitute Species

Policies and practices surrounding dog research also have implications for members of other species that might be substituted for, or replace, laboratory dogs. Policies that restrict research on dogs would in many instances transfer the burden to other large animal models, such as pigs, sheep, and non-human primates. Therefore, any ethical policy must address the ethics of substitution. The use of any animal model presupposes that a human model is not ethically appropriate because the best model for human research is, of course, a human. It must also be determined that in vitro and in silico methods are insufficient and that conducting clinical studies in companion dogs (where the veterinarian bears care obligations and the owner can provide a type of surrogate consent) rather than laboratory dogs is not feasible or scientifically appropriate.

In determining whether to use a dog or other animal, the committee believes that the most important consideration is whether their biological characteristics make them the most suitable model for the question being addressed. For example—and as discussed in detail later in this report—while the coronary anatomy of the pig makes this species a favored model for studying

atherosclerosis, the pig may be unsuitable for studying electrophysiological aspects of arrhythmia due to significant physiological differences in the Purkinje fiber networks between pigs and humans.

But physiological and anatomical characteristics are not the only criteria that should be considered. Behavioral or cognitive characteristics may make an animal more or less suitable for use in research. A non-human primate's superior cognitive ability may cause it to react differently than a non-primate, and in that context an animal that is likely to experience less harm may be a superior model, provided that the model's biological characteristics are sufficiently similar to those of humans to address the research question. A sheep may be more docile than a goat and therefore better for one study, while it may be less trainable and therefore worse for another. Even if an animal is biologically and behaviorally appropriate, if members of the species cannot be housed and handled in a way that does not induce undue suffering, they are not suitable models. Moreover, some animals may pose hazards to the research team that make them inappropriate models.

Another criterion is which species has provided the best, most relevant historical data. Past experience with a given species may support linking data from an experiment to historical data and thus allow for fewer animals to be used than would be needed in the transition to a new model. Nonetheless, this argument is potentially problematic, as it may be employed to justify the continued use of a less suitable model because the latter was used first.

There are no clear moral grounds for arguing that dogs have a "higher" moral status than other large animal species such as pigs or that scientists ought to be more restrictive about research on dogs than pigs because the former have greater moral value. What matters most in selecting a particular species, from an ethical standpoint, is that (1) the biological characteristics of the non-human animal indicate that the model will provide valuable knowledge; and (2) the harm experienced by the animals is minimized (Rachels, 2004). Some animal research protocols may be less harmful when conducted in dogs than in other species such as pigs. Others might be the reverse. For example, anxiety associated with blood draws, ureteral catheterizations, or other procedures might be less burdensome for trained dogs than for pigs. On the other hand, pigs might fare better than dogs in studies that require prolonged isolation.

Rights and Interests of Patients

Much of the biomedical research conducted with laboratory dogs is aimed at human clinical applications (exceptions include research on veterinary applications, application of human therapies in veterinary patients, and, to a lesser extent, basic research). Which research uses dogs—and how the research is performed—has clear implications for the interests and welfare of patients who might ultimately benefit from the information gathered in dog studies. In this respect, patients and other information users have interests that rival those of dogs or substitutable species.

There are several features of patient interests, however, that can be contrasted with those of dogs or substitutable species. First, whereas harms to laboratory dogs associated with a given research protocol are immediate and certain, benefits to patients are prospective and probabilistic. Not all studies advance patient care, and even when they do, many years can elapse between an experiment and its clinical application. Second, whereas harms to laboratory dogs are unconditional (they are, in a sense, hard-wired into the protocol), benefits to patients are conditioned on several factors. For example, the research needs to be designed and conducted in a way that supports valid clinical generalization, minimizing bias and confound. In order for research to advance patient care it must be disseminated in a timely manner, through complete and unbiased reporting. And there must be a community of researchers who are able to invest the time and resources in further developing a treatment approach.

There is also the circumstance in which the patient is the dog and the research using dogs supports therapeutic opportunities for both human and veterinary patients. For example, VA

investigations using laboratory dogs to find new treatments for human arrhythmias and cardiac failure also benefit veterinary treatments for arrhythmias and cardiac failure in dogs.

The Role of Societal Preferences in Establishing Ethical Animal Care Standards

Humans and dogs evolved symbiotically over tens of thousands of years. Approximately 38 percent of American households owned a dog in 2016 (AVMA, 2018). Many members of the public have an emotional aversion to the use of dogs in research that is different than their reaction to the use of other animals. While most Americans favor humane research practices for all animals in research, many would likely oppose using dogs in research. This is not surprising; many Americans love their dogs and consider them to be family members. Perhaps because farm animals are a dietary staple for many, and most Americans have very limited interactions with them, a close attachment is not common.

The extent to which societal preferences translate into ethical guidance for research policy is not entirely clear. These preferences find expression in laws that restrict and regulate the use of dogs in research (see Chapters 1 and 3 for recent legislation regarding VA research using dogs). Beyond that, it might be argued that the unique relationship with dogs obliges humans to create exceptions that favor the use of other animals over dogs when all (or most) other considerations are equal. It might also be argued that federal agencies such as the VA could show respect for views that are prevalent among the taxpayers that fund them.

On the other hand, the preference for dogs can also be viewed as prejudicial. That is, insofar as honoring such a preference would substitute other species for dogs, it discounts the welfare and interests of other species merely because many individuals prefer dogs. Most accounts of animal ethics reject "speciesism" (i.e., the notion that it is acceptable to discriminate against some animals based on their species membership), or at least they place strong constraints on it. By analogy, many people have strong bonds with members of their own culture or religion, and this finds expression in contributions to various cultural or religious charities. Few would argue that such preferences—however justified—would provide a sound basis for establishing policies that discount the welfare of members of non-majoritarian cultures or religions in terms of hiring, access to medical care, or use in research. Similarly, the moral relevance of the human–dog unique relationship is highly debatable.

Individual relationships entail special obligations, such as those owed by parents to their children, teachers to pupils, or physicians to patients. A dog owner has a stronger moral obligation to his or her own dog than to the sheep (or dog) on a neighbor's farm. It is entirely unclear, however, whether the entire population of a species (e.g., human beings) bear such moral duties. It is not clear that people in general are morally justified in discounting the welfare and interests of sheep. Nor is it clear that the entire population of a species (e.g., dogs) have moral status per se or that they have special claims that oblige humans to protect or prioritize the interests of dogs over those of other species.

According to prevailing accounts of animal ethics in the scholarly literature, there is no basis for discounting the interests and welfare of animals like sheep or pigs relative to dogs. Various commentators have derived a rejection of such speciesism in different ways. Utilitarians note that animals have interests and a capacity for suffering and there are no a priori reasons to posit that the welfare of animals counts less than that of human beings (Singer, 1975). Some argue that animals have inherent value, owing to their interest in avoiding pain and satisfying preferences (Korsgaard, 2011). Others argue that from an evolutionary standpoint, there are no principled ways to draw a moral boundary around species, because mental traits in human beings that are believed to confer moral status are present to some degree in at least some non-human animals (Beauchamp and Frey, 2011; Bekoff, 1998; Rachels, 1990). In all of these frameworks the animal's moral value is

intrinsic—it is not determined by the extent to which others assign value to the animal. In this view, the fact that members of one species are loved more than members of another species is irrelevant with respect to the duties we owe to a given animal (Regan, 1983).

The rejection of speciesism is also implicit in the pragmatic framework for animal research ethics offered by DeGrazia and Sebo (2015). One of the conditions they stipulate is the notion that no experiment should entail unnecessary harm, where necessity is dictated by the purpose of the experiment. On this view, using a species that would experience greater welfare loss for a given research objective would count as unnecessary harm. Just as the decline in welfare of one human being ought to have equal weight to the decline in welfare of another human being when considering whether an act is ethical, a decline in welfare of one animal (a human being, or a dog) ought to have identical weight to the equivalent decline in welfare in another animal (e.g., another dog, or a sheep).

Rejecting speciesism does not logically imply that all animals should be treated exactly the same. Different animals require different things to flourish. Some animals need certain kinds of bedding, others need certain forms of mental stimulation, and others need space to roam. A rejection of speciesism implies that equal consideration be given to the equivalent interests of different animals and members of different species (DeGrazia, 1996).

Institutional Considerations

As described earlier in this chapter, the use of dogs in biomedical research remains controversial. Therefore, the mission of any institution that sponsors dog research is potentially threatened by the political fallout associated with conducting such research. If negative publicity leads to diminished budgetary support or reputational harm, it can set back all parties that depend on the VA. On the one hand, every dollar that the VA spends on public relations to shore up support for dog research is one less dollar available to advance the health of veterans. On the other hand, the use of dogs to develop effective treatments can improve the VA's reputation and support while serving its core mission.

To what extent should reputational considerations drive policy at an institution like the VA? As argued above, the substitution of laboratory dogs with alternative species that would experience greater welfare loss would be unethical. In this case, any reputational benefit accruing to the VA as a result of privileging dogs would be at the expense of ethical integrity, insofar as alternative species were used. Institutions that conduct research on dogs would seem to have special obligations to employ active and effective public communication strategies aimed at educating the public to secure the necessary support and minimize reputational risk.

Synthesis

Because of the unique bond that many human beings have with dogs, it is tempting to infer that dogs bear a higher moral status and therefore deserve greater protection than other species like rabbits or pigs. However, it is important to look closely at the basis for these preferences and to ask whether they are prejudicial. Pigs, sheep, and cows—and mice and rats, for that matter—all have moral status; they have feelings, preferences, interests in their own welfare, and the ability to suffer. In varying ways, all can experience a flourishing life. A human preference for dogs does not of itself justify a lower regard for the welfare of non-dog species. By analogy, all parents have a special relationship with their own children. However, that does not make it permissible for them to mistreat other children. The welfare of all children should be held in equal regard, regardless of parentage.

If dogs should be afforded greater protection, it is not because of their superior moral status but because of how humans perceive them. Current societal preferences in the United States implicate

multiple stakeholders, and while moral status may not dictate preferential treatment for dogs, other interests may.

There was division among committee members as to whether and the extent to which dogs should receive preferential treatment in research. A majority of the committee members believe that moral status should be the principal concern and that societal interests should serve, at most, as a tie-breaker. For example, imagine that a proposed study could be conducted in pigs as well as in dogs, a careful risk–benefit assessment concludes that either would produce an equivalent gain in knowledge, and both would entail roughly the same degree of burden on the animals. In this circumstance, societal preferences could serve as a tie-breaker and justify the use of the alternate species (i.e., the pig) in research, instead of the dog. The full committee believed that such equivalences are likely to be uncommon.

In contrast, a minority of committee members do not believe that such an approach sufficiently recognizes the societal preferences at play. While they recognize the importance of the moral status of all animals, they support a position that uses dogs only when no other animal model can be used to answer the scientific question posed, even if that model is associated with much greater impact in a non-dog species.

Conclusion 2-2: Many people have a unique relationship with dogs. This relationship stems from tens of thousands of years of joint history and the cultural value of the role of dogs as service, military, law enforcement, and working partners as well as companion animals. This cultural preference for dogs is not universal, nor does it necessarily constitute a reliable guide to ethical action. The majority of the committee concludes that it is valid to consider the societal preference for dogs only in situations where expected burden for substitute species is anticipated to be equivalent to that projected for the laboratory dog.

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3

Determining the Necessity of Laboratory Dogs in Biomedical Research Funded by or Conducted at the U.S. Department of Veterans Affairs

This chapter addresses the committee's task (see Chapter 1, Box 1-1) to explore recent past, current, and anticipated research questions directly related to the mission of the U.S. Department of Veterans Affairs (VA) to determine if laboratory dogs are or will continue to be necessary for future VA biomedical research.

The chapter begins with an overview of dog use in biomedical research in the United States. This is followed by a consideration of laboratory dog use in 10 biomedical research fields related to the VA's mission, including seven areas in which the VA currently uses laboratory dogs or has done so in the recent past (cardiovascular disease [CVD], spinal cord injury [SCI], imaging, diabetes, narcolepsy, chronic pain and osteoarthritis [OA], and experimental pharmacology and toxicology) and three areas of potential future use (cancer, infectious disease, and Alzheimer's disease). Six other areas of biomedical research in which the VA has used dogs, some in the past decade, are also discussed briefly.

The committee's efforts focused on exploring areas of biomedical research using laboratory dogs in the VA's current and recent portfolio (2016 onward) and, to a more limited extent, areas relevant to the VA's mission where dog use may be considered in the future. It would not have been feasible for this committee to cover all possible research areas of interest to the VA, and the absence of a particular field from this report should not be taken as a determination regarding the necessity of dog use in that field.

The chapter concludes with a discussion of the committee members' various interpretations of "necessary" and the committee's recommendations, including dissenting opinions, for guiding the VA's determination of when laboratory dogs are necessary for biomedical research (see Recommendations 1 and 2). The committee also provides a recommendation for improving the VA's biomedical research protocols and review processes (see Recommendation 3).

TRENDS IN DOG USE IN U.S. RESEARCH FACILITIES

Animals, including dogs, have been used in scientific demonstrations, teaching, and research since antiquity (Kinter and DeGeorge, 2016). Dog use increased dramatically in the late 19th and early 20th centuries, paralleling the development of increasingly sophisticated instrumentation for measuring physiological function, along with advances in analytic and organic chemistry that led to an explosion in the synthesis of small molecules requiring evaluation of their pharmacological properties[1] (Kinter and DeGeorge, 2016). These trends in molecule synthesis and animal use continued through the mid-20th century and were amplified by new considerations, including requirements for animal safety testing in order to obtain government approvals for clinical trials and marketing of regulated products and the growth and proliferation of international biomedical research stimulated by post–World War II industrial expansion. Several members of this committee recall the large number of sophisticated dog models that supported basic human and veterinary physiological research in the postwar decades, as well as dog bioassays for pharmacological and toxicological research and product discovery and development. The advent of new molecular techniques in the 1970s and 1980s likely played a role in replacing animal research models, particularly dogs. Data from U.S. Department of Agriculture (USDA) annual reports tracking dog use from 1973 to 2018 are illustrated in Figure 3-1. (See also Appendix B for further analysis of the USDA data.)

The specific factors leading to decreased dog use since the 1970s are uncertain but could include the advancement of molecular techniques, societal pressures and preferences, and the high cost of using dogs in biomedical research. It is also the case that while there has been a notable decrease in dog use since the 1970s, dog use in the past decade has been steady at around 60,000 dogs per year.

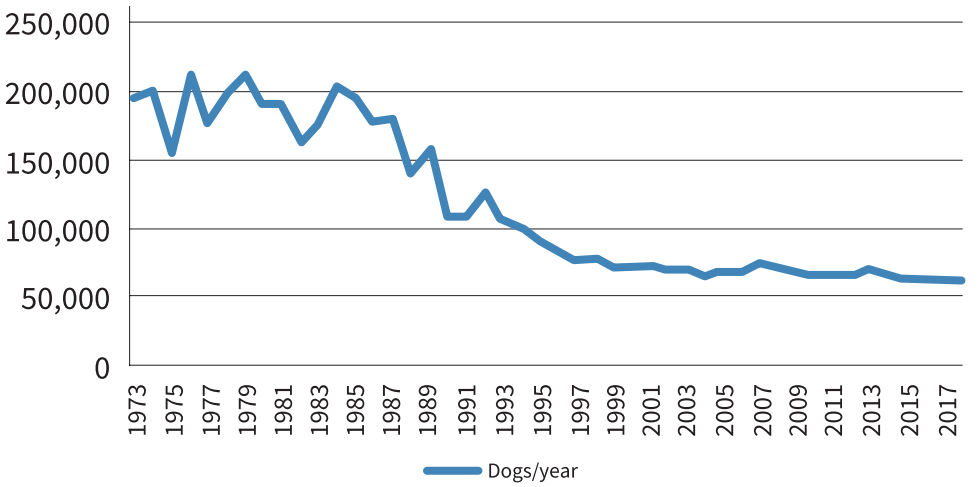

FIGURE 3-1 Annual dog usage in the United States from 1973 to 2018, based on data collected by the U.S. Department of Agriculture.

[1] L. B. Kinter, personal communication, November 6, 2019.

Current Distribution of Laboratory Dog Use in U.S. Research Facilities

The committee reviewed the 1,149 annual reports submitted to USDA's Animal and Plant Health Inspection Service[2] from research facilities in all 50 states and territories in 2017 (the most recent year with complete publicly available data). This review indicates that a total of 60,190 dogs were used in the United States in 2017, of which 22,933 were used by 213 academic institutions and affiliated hospitals engaging in biomedical research and education (including veterinary research conducted for the benefit of dogs); 34,875 by 105 companies and private research organizations engaging in applied biomedical research and human and veterinary product development (industry), including testing required by regulatory agencies; 832 by 11 government agencies (including VA research labs) conducting basic and applied research; and 1,550 by 16 other, non-research groups. USDA data indicate that industry is currently the dominant user of dogs for biomedical research, with the use of dogs by industry exceeding the usage by academic institutions, government, and non-research groups combined.

LABORATORY DOG USE IN BIOMEDICAL RESEARCH AT THE VA

The VA provided the committee with documentation from 44 research projects involving dogs conducted by its researchers over the past 50 years. Of these projects, 30 dated from the 1960s to 2017 and 14 were active in 2018–2019 (VA, 2018a,b). Among these studies, cardiovascular/renal was the most frequently cited application (17), followed by central nervous system (15), endocrine (4), respiratory (4), gastrointestinal (3), and animal/surgical models (4). Some VA projects covered more than one research area, hence the total number of areas exceeds the number of projects. One VA study, undertaken to test a treatment for a spontaneous melanoma that occurs in both dogs and humans, used companion rather than laboratory dogs (VA, 2018b). In research performed at or funded by the VA, 99 percent of animals used in 2017 were mice or rats, while fewer than 0.05 percent were dogs (VA ORD, 2018).

In the following three sections, the committee surveys the use of dogs in 10 biomedical research areas—CVD, SCI, imaging, diabetes, narcolepsy, chronic pain and OA, experimental pharmacology and toxicology, cancer, infectious disease, and Alzheimer's disease. The purpose of this overview is to establish a context for assessing both the current need for laboratory dogs in each of these fields and the likely need for dogs in future VA biomedical research. CVD, SCI, and imaging are the current areas of laboratory dog use at the VA. Diabetes, narcolepsy, chronic pain and OA, and experimental pharmacology and toxicology are areas in which dogs were recently used (since 2016), and the remaining three fields (cancer, infectious disease, and Alzheimer's disease) were chosen based on their potential for future use (primarily in companion dogs). As stated in the introduction to this chapter, the absence of a particular field of research should not be taken as a determination regarding the necessity of dog use in that field.

Research Areas with Current Laboratory Dog Use at the VA

This section describes the state of research in three areas of current laboratory dog use at the VA—CVD, SCI, and imaging. The committee reviewed current practices and recent advancements

[2] The Animal Welfare Act of 1966 (U.S. Code 7 Chapter 54: Transportation, sale, and handling of certain animals) mandated that USDA collect annual reports listing the numbers of vertebrate animals (excepting mice, rats, and birds) used by all USDA registered academic and industrial research facilities as well as federal research facilities, including the VA. Reports from individual facilities are publicly available and organized by state at https://acis.aphis.edc.usda.gov/ords/f?p=118:205:0 (accessed December 10, 2019).

in each of these areas of research to better understand the context for the VA's research using laboratory dogs in these areas.

Cardiovascular Disease

CVD encompasses numerous clinical entities and is the leading cause of death in the U.S. population (NCHS, 2017) and globally (Mendis et al., 2011). In a 2014 longitudinal study, the U.S. veteran population was shown to be at increased risk for the development of CVD (Assari, 2014), and veteran status was identified as a risk factor independent of other co-factors and comorbidities. A website maintained by the VA's Office of Research and Development identifies CVD as the leading cause of hospitalization in the VA health care system and as a major cause of disability (VA ORD, n.d.a). The reader may refer to this site for additional information regarding the current status of VA cardiovascular research programs as well as their historical contributions to the advancement of clinical care, discovery, and scholarship.

The laboratory dog has a long history of use as an experimental model for the investigation of cardiovascular physiology and disease. Early work in dogs led to notable advances in the understanding of the role of the autonomic nervous system in the regulation of heart rate and blood pressure (Halsey, 1917), the renin–angiotensin–aldosterone system in the control of blood pressure and circulatory volume (Goldblatt et al., 1934; Watkins et al., 1976), the pathogenesis of hypertension related to renal vascular disease (Goldblatt et al., 1934), cardiac electrophysiology and conduction disorders (Ross and Franklin, 1976), congenital and acquired heart diseases and their surgical correction (Shumway et al., 1962; Toledo-Pereyra, 2010), and congestive heart failure (Conn et al., 1966; Watkins et al., 1976).

In the development of modern cardiovascular care, from the latter half of the 20th century to the present, almost every major milestone has involved dogs. Dogs played a role in development of the pacemaker, the internal cardiac defibrillator, angioplasty, stents, congenital heart surgery, valve surgery, transplants, and transcatheter aortic valve replacement (Bernstein, 2019), and they continue to be used in efforts aimed at improving these technologies.

Nonetheless, the 1980s saw the beginning of a decline in the use of dogs in research (see Figure 3-1), including for cardiovascular research, as many disciplines shifted to other animal models, particularly rodents, which were better suited to investigations of the genetic and molecular mechanisms of disease. The miniaturization of technology and the adaptation of techniques to rodents, combined with the increased use of alternative large animal models, accelerated the decline in dog use. Over time, these alternative models—particularly the pig, goat, sheep, and non-human primate—have come to share the cardiovascular research landscape with the laboratory dog.

A survey of eight major cardiovascular journals over the past 20 years found that most studies reporting experimental models used rodents, primarily mice (Harrison, 2019). Rodents were the most commonly used animal model for studying hypertension, atherosclerosis, heart attack, heart failure, obesity and diabetes, and other vascular diseases. However, not all questions can be adequately addressed in rodents, due to their smaller size and anatomical and physiological differences from humans, and large animals frequently serve as a bridge for translating new findings from rodents to human patients.

The choice of which large animal model to use hinges on the model's similarity to the particular human condition being studied. Pigs are now favored over dogs for coronary artery disease research, for example, because of the greater similarity of the pig's coronary anatomy to that of humans (Vilahur et al., 2011; Weaver et al., 1986). Atherosclerotic coronary artery disease is readily inducible in the pig (Granada et al., 2009) and atherosclerotic plaques develop in locations relevant to the human condition (Brodala et al., 2005; Hasler-Rapacz et al., 1995) and lead to metabolic syndrome (Myers, 2019). A review of the most common large animal models for studying heart

disease, which addresses the particular biological properties that may favor selection of one model over the others for a given investigation, was published recently (Camacho et al., 2016).

Despite the increase in the use of rodents, pigs, and sheep, dogs remain a preferred model for studying certain aspects of CVD. The committee's review of the scientific literature describing cardiovascular research in dogs from 2009 to mid-2019 turned up hundreds of publications, indicating that the laboratory dog continues to serve as an important animal model for CVD, both in the U.S. research enterprise and globally. Dogs were primarily being used as models for the investigation and treatment of cardiac arrhythmias and congestive heart failure and for the development and validation of devices used in the diagnosis or therapy of CVD. The following discussion summarizes the salient trends revealed by this literature survey.

Aspects of arrhythmia The laboratory dog is used extensively for studying both atrial and ventricular arrhythmias, specifically for interrogating those physiological processes for which alternative animal models are less suitable. Rodent heart rates (500–700 bpm) and vascular shear rates differ dramatically from those of humans and dogs (Harrison, 2019; Suo et al., 2007). The ratio between the size of the myocardium and wavelength is critical for establishing the pathophysiology of arrhythmia; the animal in which that ratio is most similar to that of humans is the rabbit, followed by the dog and the pig (Efimov, 2019; Panfilov, 2006). Combined with the fact that the pig's Purkinje system differs from the human one, this similarity has supported the argument that the pig is a poor model for arrhythmia, at least in terms of modeling human pathophysiology. Nonetheless, pigs are used in many aspects of arrhythmia translational research. A recent analysis recommended pigs as the primary choice for studying myocardial ischemia and atrial tachycardia and suggested further research to characterize pigs as models for ventricular tachycardia (Clauss et al., 2019). Dogs may nonetheless be preferred for certain investigations, such as evaluation of the autonomic modulation of arrhythmias (Piktel and Wilson, 2019).

For atrial fibrillation (AF), the most common type of clinical arrhythmia in humans, the dog model involves the induction of AF through atrial tachy-pacing at a rate of 400 bpm for an extended period. This model is currently used in the investigation of diverse topics in AF, including atrial electrocardiogram analysis (Gerstenfeld et al., 2011); defibrillation therapy (Janardhan et al., 2014; Witt et al., 2018); atrial remodeling (Nakatani et al., 2013; Yamashita et al., 2019); the role of the autonomic nervous system (Ardell et al., 2014; Katsouras et al., 2009; Nishida et al., 2011); genetic, molecular, and channel biology studies (Shorofsky et al., 2009; Wakili et al., 2010; Wang and Li, 2014; Wei et al., 2018); and the evaluation of antiarrhythmic drugs (Qi et al., 2014; Sakabe et al., 2012). Research into new approach methodologies in AF has advanced in recent years and holds promise for some future cardiovascular research efforts to move away from dogs (see Chapter 4 for discussion of alternatives). Canine tissues and cells—either isolated from tachy-paced dogs or tachy-paced in vitro—are also frequently employed (Aguilar et al., 2014; Makary et al., 2011; Wiersma et al., 2017). Thromboembolism and stroke are major complications associated with atrial enlargement in AF patients, and these are managed pharmacologically with anticoagulation therapy and beta blockers. The laboratory dog is being used for the development and evaluation of nonpharmacological strategies for stroke prevention, including minimally invasive surgical procedures and new devices (Bruce et al., 2011; Fumoto et al., 2012; Hill and Guy, 2012; Kar et al., 2014; Lee et al., 2010b; Schwartz et al., 2010; Sunagawa et al., 2017).

The laboratory dog has also retained a prominent position in the study of ventricular arrhythmias, which may be attributable to several factors. The dog's Purkinje fiber network has histological (Ono et al., 2009) and electrophysiological (Huang et al., 2014) characteristics that are more similar to the human than are those of the pig (Efimov, 2019). The dog is also very sensitive to the development of electrocardiographic prolongation of the QT interval, which is regarded as an early potential predictor of Torsades de Pointes, a severe polymorphic ventricular arrhythmia that

may cause sudden death. For this reason, the laboratory dog has frequently been the in vivo model of choice in the deselection of new pharmacological compounds. It should be noted, however, that the currently available bioassays for proarrhythmic activity, including the dog, are not ideal (Lee et al., 2010a). Dogs are more sensitive than humans to the development of prolonged QT, which may lead to the disqualification of potentially useful drugs. This has led some researchers to pursue new electrophysiological parameters and other approaches that may improve the predictive value of the dog model (Boulay et al., 2019; Marostica et al., 2016; van der Linde et al., 2010; Vargas, 2010).

Canine myocardial infarct models are widely used for the study of ventricular arrhythmias, where the influence of the spinal cord and autonomic nervous system on arrhythmogenesis and pursuit of new therapeutic approaches have become objects of intense focus (Baburin et al., 2018; Chen et al., 2013, 2016; del Rio et al., 2015; Lopshire et al., 2009; Nasi-Er et al., 2019a,b; Wang et al., 2015; Zhang et al., 2018; Zhou et al., 2019). The protective effects of nutritional factors (Bonilla et al., 2016), exercise (Billman, 2009; Kukielka et al., 2011), intermittent hypoxia (Estrada et al., 2016), and new compounds (Lee and Lucchesi, 2013) have been investigated in the canine myocardial infarct model.

Cardiomyopathies Dogs are also used to model nonischemic congestive heart failure and cardiomyopathy (Camacho et al., 2016; Dixon and Spinale, 2009). Cardiomyopathy occurs naturally in several dog breeds, including Doberman pinscher, Newfoundland, Irish wolfhound, boxer, and golden retriever, and this may prove useful for future investigations of genetic, molecular, and environmental factors common to canine and human cardiac diseases (Kaplan et al., 2018; Meurs, 2017; Meurs et al., 2012; Simpson et al., 2015, 2016; Vischer et al., 2017; Wiersma et al., 2008).

Device testing In addition to its use for the development of procedures and devices aimed at treating arrhythmias, the dog remains an important model for the development and evaluation of devices used in other areas of cardiovascular diagnosis and therapy. For testing intravascular devices, which require stable vascular dimensions over time, the dog and sheep (Joscht et al., 2016; Kalder et al., 2019) have a distinct advantage over the pig, due to the pig's continuous body growth (and commensurate changes in vasculature) throughout its life span (Myers, 2019). Dogs have been used for more than 30 years for the evaluation of intravascular stents to repair vascular defects and restore blood flow (Jeremy and Thomas, 2010; Sigwart, 2017), and there are numerous examples in the most recent decade of the contributions of the dog model to the understanding of stent design, improvements in biocompatibility, and reductions in complications (Bastijanic et al., 2014; Cho et al., 2014; Kawajiri et al., 2015a,b; Lequoy et al., 2016; Li et al., 2019; Martinez Moreno et al., 2019; Paul et al., 2012, 2013; Watanabe et al., 2014; Zhang et al., 2015). Intracardiac devices to address valvular, shunt, and septal defects have also been studied in the laboratory dog (Gruenstein and Bass, 2009; Takaseya et al., 2010). Research on the endograft model for repair of aortic aneurism switched to a sheep model as part of the general movement away from dogs. However, there have been recent reports of late failure of this device in humans, which is likely to require investigation in an animal model. This may lead to a return to dogs for studying the larger pelvic implant, which is well tolerated in dogs but causes paralysis in sheep (White, 2019; White et al., 1996; Wilson et al., 1997).

Pacemakers have been used to control heart rate in humans since their development in a laboratory dog model over half a century ago. The first pacemaker ever placed in a human, in 1958, lasted 9 hours (Bernstein, 2019). The continued advancement of this technology, as well as the development and improvement of intravascular defibrillators and the combined cardioverter–defibrillator, has relied on the laboratory dog (Bryant, 2017; Merkely et al., 2013; Sanders et al., 2011), although it has also used pigs in many instances. Dogs are also widely used to evaluate new approaches and devices for supporting the heart during cardiac failure, including ventricular

assist devices and cardiac restraint devices (Clarke et al., 2015; Kakino et al., 2017; Kubota et al., 2014; Sabbah et al., 2009; Saku et al., 2016). Devices that enable an endovascular approach to denervation (Jordan et al., 2012), tissue ablation (Jilaihawi et al., 2010), central nervous system interventions (Kara et al., 2014), and protection from embolism (Kara et al., 2014) have also relied on the laboratory dog.

VA cardiovascular disease research using laboratory dogs The VA has used dogs since the 1960s to investigate the consequences and treatment of cardiac rhythm disorders and heart failure. Early VA investigations using the dog resulted in the development and routine clinical use of the pacemaker to stabilize heart rate in humans (Chardack, 1964; Chardack et al., 1960, 1962, 1963). The size and electrophysiological similarity of the dog heart to the human heart established the dog as the preferred model for those studies. Subsequent VA researchers have cited additional similarities between human and dog collateral coronary circulation, heart geometry, heart rate, and autonomic nervous system as important features for their cardiovascular studies. Between the 1980s and 2000s VA research teams extensively studied the mechanisms for heart failure and pharmacologic approaches to intervention (Carabello et al., 1992; Ishibashi et al., 2001; Ishihara et al., 1992; Matsuo et al., 1998; Nagatsu et al., 1994, 2000; Nemoto et al., 2002; Tsutsui et al., 1994; Zile et al., 1991). Also during this period, VA researchers developed numerous techniques and equipment for the ablation of cardiac arrhythmias in humans (Antz et al., 1998, 2001; Hasdemir et al., 2003; Jackman and Zipes, 1982; Jackman et al., 1988; Nakagawa et al., 1998; Schauerte et al., 2000; Wittkampf et al., 1996).

More recently, VA researchers have relied on the dog to investigate the reasons for the failure of surgical ablation to eliminate AF in some cases (Melby et al., 2008) and to develop other novel approaches for the treatment of AF (Chinda et al., 2016; Ruaengsri et al., 2018). Clinical observations in patients also led VA researchers to investigate problems associated with premature ventricular contractions (PVCs) in the dog. These studies established that, in some cases, PVCs induce cardiomyopathy (Huizar et al., 2011), and this was subsequently recognized as a distinct clinical entity by the American Heart Association (Al-Khatib et al., 2018). A search for strategies to prevent sudden cardiac arrest found that certain electrocardiographic signals, called T-wave alternans, correlate with the onset of potentially lethal ventricular arrhythmia (Kwofie et al., 2011). Recent publications from the VA demonstrate their ongoing interest in understanding the cellular and molecular changes underpinning the development of PVC-induced cardiomyopathy as well as the neural mechanisms that might be useful for the reduction of PVCs (Gunda et al., 2019; Huizar et al., 2019; Jiang et al., 2016; Tan et al., 2016; Wang et al., 2014).

Summary While laboratory dogs have been supplanted by rodents for most CVD research, there remains extensive ongoing research using laboratory dogs to investigate several topics relevant to human CVD. Laboratory dogs continue to be used for the study of both atrial and ventricular arrhythmias, owing to physiological traits (heart rate, vascular shear rate, myocardium/wavelength ratio, Purkinje fiber network, etc.) that model the human state better than do other large animal or rodent models. Laboratory dogs also remain important for device testing and improvement, specifically for those devices that cannot be tested in sheep or pigs owing to either physiological differences or the need for long-term stability in vivo. As described in Chapter 4, pigs are seeing increasing use for research into myocardial ischemia and atrial tachycardia, and they are beginning to be characterized as models for ventricular tachycardia. The availability of genetically engineered minipigs is likely to increase the trend away from laboratory dog research for CVD, until sufficient new approach methodologies are available.

The natural occurrence of CVD in multiple breeds also makes companion dogs promising candidates for the investigation of the genetic and environmental factors that influence heart fail-

ure. Indeed, veterinary care of companion dogs commonly includes treatment for a range of CVDs (arrhythmia, hypertension, heart failure, etc.). Research using companion dogs stands to benefit both dogs and humans.

Spinal Cord Injury

There are 291,000 people in the United States with an SCI, 59.9 percent of which are cervical injuries resulting in tetraplegia and loss of natural breathing (NSCISC, 2019). An additional 39.5 percent are thoracic injuries that cause paraplegia (NSCISC, 2019). Health concerns of individuals with SCI are severe and include loss of hand and arm function, pressure sores, loss of bowel and bladder function, urinary tract infection, impaired breathing and cough, spasticity, neuropathic pain, and loss of sexual function (Alilain, 2019; Floyd, 2019). There is currently no cure for SCI and no therapeutic to improve outcome other than rehabilitation (although epidural stimulation has generated some promising results, as described below). The VA cares for more than 27,000 veterans afflicted with SCIs and related disorders annually (VA ORD, n.d.b).

The use of laboratory dogs as research models for SCI has a long history that goes back to the first direct experimental contusions in 1911 (Allen, 1911). Over the past half-century, however, much SCI research that previously involved laboratory dogs (as well as cats and rabbits) shifted to rodents. Rodents are now the preferred species for the initial evaluation of therapies aimed at facilitating spinal cord repair, although the human injury can be more closely approximated in large animals (Cheriyan et al., 2014). While research aimed at neuroprotection and regeneration has produced encouraging results in rodents (MacFarlane et al., 2018; Warren and Alilain, 2019; Warren et al., 2018), it has failed to generate any effective pharmacologic or stem cell approaches for treating humans to date (Alilain, 2019; Floyd, 2019), despite claims to the contrary by nonregulated stem cell "clinics" (Gabel et al., 2017).

Translational spinal cord injury research Despite the predominance of rodents in SCI research, taking a treatment straight from rats to humans raises serious concerns. A large animal, such as a pig or dog, constitutes an intermediate model that increases confidence in the ability to safely translate a treatment from laboratory to clinic. Large animals are more similar to humans in terms of size, brain anatomy, blood flow, pharmacokinetics, and the complexity of the spinal circuitry (Floyd, 2019), and they are more amenable than rodents to detailed locomotor assessment, electrophysiology, bladder function tests, and high-quality brain imaging (Jeffery, 2019). Furthermore, *not* conducting large animal studies risks serious adverse events in a first-in-human study, with the potential not only to harm the patients involved but also to derail the development of an important therapy (Guest, 2019). While translational SCI research in laboratory dogs has a long history, few effective treatments for SCI currently exist, leaving significant opportunity for enhanced collaboration among researchers in this area and exploration of companion animal and new approach methodologies.

Use of companion dogs to study dog thoracic spinal cord injury There is a growing body of research into the restoration of motor function which employs a variety of interventions in large animals. This research has seen considerable growth in the realm of veterinary medicine, aided by the relatively high incidence of SCI in companion dogs and the willingness of many pet owners to "volunteer" their affected dogs for research that may offer a therapeutic benefit for the dog. Although the treatment of the dog's SCI is the primary aim of these studies, the findings have potential to inform the treatment of analogous lesions in humans. A canine SCI consortium, CANSORT-SCI, was founded to leverage this opportunity, using clinical trials performed in companion dogs for the benefit of dogs, to facilitate the translation of results to humans (Moore, 2019; Moore et al., 2017). An international canine SCI observational registry has been established, and its founders argue

that companion dogs' heterogeneity with respect to both injury and genetic backgrounds offers an advantage in the way it parallels the diversity of human injuries and genotypes (Moore et al., 2018). Veterinary clinical trials for SCI are enabling therapies that were found to be effective in rodents more than a decade ago to be tested for the first time in large animals (Granger et al., 2012; Hu et al., 2018). It is important to note, however, that the injuries studied in companion dogs are thoracic, which represent the minority of human SCI injuries.

Device testing Another approach to treating SCI employs devices to drive essential life processes, such as breathing by selective electrical stimulation, which seeks to enable patients with cervical SCI to survive without mechanical ventilation despite the persistence of the injury. Preclinical research into device-driven breathing is carried out in large-animal models, including dogs, and was the topic of the SCI studies involving laboratory dogs at the VA published in 2018–2019 (DiMarco and Kowalski, 1985; Kowalski et al., 2019). These experiments, designed to restore respiratory muscle function, recently produced evidence of successful translation from dogs to humans (DiMarco et al., 2019a). Beyond the VA, another example of an SCI device-dependent intervention that has been moved from animal studies into humans is epidural stimulation in combination with physical therapy, which has restored some patients' ability to walk (Harkema et al., 2011; Wagner et al., 2018).

The extent to which preclinical SCI research in animals can predict human outcomes remains an open question. Numerous studies have revealed the complexity of SCI and the multiple variables that need to be addressed in order for effective interventions, aimed at repairing the damage, to have a chance at success. In addition to the obvious anatomical differences among species, there is divergence in spinal tract reorganization and functional recovery (Friedli et al., 2015). There is also a discrepancy in focus, with 81 percent of 2,209 published animal studies (1946–2016) focusing on thoracic SCI, whereas cervical SCI is the more common injury in humans (Sharif-Alhoseini et al., 2017). Of these animal SCI studies, 72.4 percent (1,599) were in rats, 2.3 percent (51) were in dogs, 2.2 percent (48) were in cats, 2.4 percent (53) were in rabbits, and 1.5 percent (33) each were in pigs and non-human primates. A review of large animal models of stem cell therapies for SCI identified 11 dog studies between 2007 and 2014, two of which used dogs with natural rather than induced injuries (Gabel et al., 2017).

Current use of dogs in spinal cord injury research A search of the PubMed database for SCI studies involving dogs published in 2017–2019 revealed that almost all of these used companion dogs with thoracic injury, including one study that harvested cells from oral mucosa to generate stem cells for transplantation into the spinal cord (Ito et al., 2019) and another that tested low-level electrical stimulation, transplantation of adipose-derived stem cells, or a combination of the two (Krueger et al., 2019). The harvested-cell study noted wide variability in stem cell yield, while the electrical-stimulation plus adipose-derived-stem-cell study failed to demonstrate the hypothesized superiority of this combination treatment. Results from both studies indicate that much more needs to be learned before the stem cell approach can be translated into humans. A 2018 review of large animal hemisection models of SCI (Wilson et al., 2018) cited two Korean studies, performed in 2009 and 2010, that demonstrated the migration of human neural stem cells into dog spinal cord tissue and functional recovery (Kim et al., 2010; Lee et al., 2009); some of this work may have subsequently been moved to a rat model (Hong et al., 2017).

The structure of the spinal cord in the pig is close to that in humans (Guest, 2019), and recent studies aimed at optimizing techniques for delivering cells to patients with chronic SCI used the Yucatan minipig as a large animal bridge from earlier preclinical studies performed in rodents and primates (Benavides et al., 2017; Casas and Guest, 2004; Kutikov et al., 2019; Lim et al., 2010). Where the companion dog model cannot be used, such as for early drug discovery studies

or for studies that require carefully timed injury intervention (Moore, 2019), the pig offers a large animal alternative to laboratory dogs. However, the continuous growth of pigs (including minipigs) throughout their life span, as well as difficulty in managing pigs, can limit their utility for long-term testing of implanted devices, while primates (non-human and human) remain essential for the testing of hand function and the role of the immune system in SCI (Floyd, 2019; Guest, 2019; Jeffery, 2019). A sheep model is also under development (Wilson et al., 2019).

VA spinal cord injury research using laboratory dogs Recent advances in the development of new devices to stimulate breathing in humans with cervical SCI, described above, represent the culmination of more than two decades of research and rely on a dog model that was developed by the same group of VA investigators (Walter et al., 2007). The researchers noted that, although pigs were large enough for the devices, anatomical differences have prevented pigs from becoming an established model for respiratory stimulation in humans. VA research performed in the 2000s used dogs to explore methods to electrically stimulate bladder control in people with SCI (Bresler et al., 2008). The VA also supported research into a cough stimulator to reduce the incidence of respiratory infections and improve the quality of life for people with SCI (Kowalski et al., 2016). This device led to improved pulmonary function among tetraplegics enrolled in a recent clinical trial (DiMarco et al., 2019b) and is now being used by veterans with SCI. Research is continuing to refine this device to help individuals with SCI and others who have difficulty coughing such as those who experienced strokes or have amyotrophic lateral sclerosis.

Summary The majority of SCI research is now carried out in species other than dogs or in companion dogs with thoracic injury, which stand to benefit directly from the development of new therapies. Pigs have been effective models for studying pathogenesis and translating therapies aimed at neuroregeneration, as described in Chapter 4. However, for a small number of investigations that are highly relevant to humans with SCI, such as those interrogating respiratory function in patients with cervical injury, laboratory dogs remain a model of choice, owing to specific anatomical and physiological features that cannot be recapitulated in other large animals.

Imaging

In the early days of imaging that followed the discovery of X-rays in 1895, use of the new technology often took a greater toll on human investigators, who had no awareness of the radiation to which they were exposing themselves, than on their canine research subjects (Babic et al., 2016; Barger, 1981). In time, radiation protections were implemented, and imaging modalities evolved from radiographs to ultrasound to computed tomography to magnetic resonance imaging (MRI), in some cases moving away from the use of radiation altogether. However, with increasingly sophisticated surgical techniques and other biomedical interventions constantly under development, dogs have been regularly called on for testing of both new treatments and new uses of imaging technology (Baumeister et al., 2016; Brooks et al., 2019; Chatal et al., 2015; Hadrian and Palmes, 2017; Millon et al., 2014).

Due to their larger size compared with rodents and to their ease of handling, laboratory dogs have frequently been used in clinical investigations to test a variety of imaging techniques. The radionucleotide rubidium-82, used to image myocardial perfusion, was first assessed in dogs, from the recognition of its similarities to potassium in 1954 through the first human injections in the early 1980s (Chatal et al., 2015). Laboratory dogs have also been used to investigate a number of other imaging techniques, including the following: cardiac MRI for the detection of reperfusion hemorrhage following experimentally induced myocardial infarction (Kumar et al., 2011), the use

of MRI and digital subtraction angiography to evaluate a model of subarachnoid hemorrhage (Mori, 2014), and the role of MRI in tracking the development of atherosclerotic plaques in experimentally induced atherosclerosis (Millon et al., 2014). Dogs are also being eyed as potential contributors to comparative neuroscience using functional MRI, where they have begun to offer insight into the way human language is represented in a non-primate mammalian brain (Andics and Miklosi, 2018).

Dogs are not, however, the model of choice for all imaging studies. There is significant interspecies diversity with respect to the safety and efficacy of imaging agents which varies with the specific class of compounds, sometimes unexpectedly. Rabbits, for example, were preferred for the development of contrast-enhanced subharmonic ultrasound (Eisenbrey et al., 2015), and pigs are useful for thrombectomy modeling and imaging (Chueh, 2013). Studies involving the inter-atrial septum of the heart are preferentially done in swine or sheep, as the canine inter-atrial septum lacks sufficient anatomic similarity to humans (Jalal et al., 2018). Efforts at imaging the lymphatic system (lymphoscintigraphy) have moved from canine to rodent models, where the circumferential excision of lymphatic tissue can induce the required secondary lymphedema (Hadrian and Palmes, 2017).

VA imaging research using laboratory dogs The narrowing of the arteries supplying blood to the kidneys can cause hypertension and kidney damage. Prior to the 1980s, the measurement of blood flow through renal arteries required a flowmeter to be surgically placed on the artery. VA research conducted on dogs in the 1980s helped validate the accuracy of blood flow measurements using non-invasive ultrasound imaging (Avasthi et al., 1984; VA, 2018b). Ultrasound is now accepted as the standard clinical method for assessing renal artery stenosis, which is estimated to affect 70,000–400,000 veterans (VA, 2018b).

Summary Although laboratory dogs were critical for the development of many imaging techniques now taken for granted and still may have a role to play in studying brain function, they no longer constitute a default model for the development or testing of novel imaging modalities, particularly in those cases where an alternative is likely to exist. Nonetheless, given the great diversity of response to imaging compounds among laboratory species, the possibility that dogs may be required for specific imaging needs in the future cannot be ruled out.

Research Areas with Recent Laboratory Dog Use at the VA

This section describes the state of research in four areas of recent laboratory dog use at the VA—diabetes, narcolepsy, OA and chronic pain, and experimental pharmacology and toxicology.

Diabetes

Diabetes has been a human scourge since antiquity (Eknoyan and Nagy, 2005). Two types of diabetes are recognized: a devastating form that primarily affects youth and a milder form that primarily affects overweight adults, termed type 1 and type 2, respectively. More than one-third of the global population is at risk of developing type 2 diabetes (Kleinert et al., 2018). Type 1 diabetes is characterized by the absence of insulin, a key regulator of body glucose utilization, whereas type 2 diabetes is characterized by cells' inability to respond appropriately to circulating insulin. Today, type 2 diabetes is associated with a constellation of dysfunctions labeled the "metabolic syndrome" in both adults and youth. Diabetes was the seventh leading cause of death in the United States in 2017, with 1.5 million new cases diagnosed that year (CDC, 2020). Diabetes affects nearly 25 percent of VA patients and is the leading cause of blindness, end-stage renal disease, and amputation at the VA (VA ORD, n.d.c).

Trends in dog use for diabetes research Dogs have played a central role in diabetes research from its earliest days. Dogs are susceptible to heritable (Cai et al., 2019), autoimmune (O'Kell et al., 2017), and environmentally stimulated diabetes (Kleinert et al., 2018) and could benefit from the same treatment strategies used in human patients. The discovery of insulin may be one of the most consequential biomedical achievements of the 20th century, not only for its impact on diabetes management in human and veterinary medicine, but for launching a century of far-ranging advances in the chemistry, biology, therapeutics, and synthesis of peptide hormones, proteins, and drugs; as well as in the manufacture of delivery systems.

With the advent of human insulins and more sophisticated technologies for insulin administration, the focus has shifted to type 2 diabetes. Dogs are susceptible to developing overweight/obesity related to diet/overfeeding but are more resistant than humans to the development of full-blown type 2 diabetes (Kleinert et al., 2018) and therefore have seen relatively little research use for this disorder compared to type 1 diabetes. Most current experiments using animals in diabetes research are carried out in rodents, with a focus on rodent models of metabolic syndrome and the glucose tolerance test (e.g., Brott et al., 2013). Nonetheless, larger animals are still used for some pharmaceutical safety studies and studies directed at veterinary care (Kleinert et al., 2018; Kumar et al., 2012).

VA diabetes research using laboratory dogs Research performed in the century since the discovery of insulin has elucidated the role played by pancreatic islet cells in the control of blood glucose and tissue glucose use. In the 1980s and 1990s the VA funded basic research on the microstructure of the islets; this work used dogs because islet microstructure in humans is similar to that in dogs and quite different from rodents (VA, 2018b). In 1992 a VA researcher helped demonstrate the successful reversal of diabetes in dogs by the intraperitoneal implantation of microencapsulated islet cells (Soon-Shiong et al., 1992; VA, 2018b). One result of this work is that the dog is now viewed as the translational model for pancreatic islet transplantation in humans (Adin and Gilor, 2017).

Insulin administration was an immediate improvement in the lives of diabetes patients. However, the injection of insulin is complicated, with patients risking hypo- or hyperglycemia if the amount given is too high or too low. The need to better regulate insulin administration stimulated research into "artificial pancreas" technology. VA research into basic physiological mechanisms of insulin regulation of blood glucose, going back to the 1980s, laid the groundwork for the development of insulin control strategies (Benthem et al., 2001; Havel et al., 1996; VA, 2018b). In the intervening years the emphasis has shifted from transplants to miniaturized mechanical insulin pumps, wearable glucose sensors, and microcomputer algorithms for the continuous control of glucose levels (Bekiari et al., 2018), circumventing the safety issues associated with implanted tissues. Collaboration between the VA and private industry (VA, 2018a) led to the U.S. Food and Drug Administration's approval of the MiniMed 670G, the first device to automatically monitor blood glucose and provide appropriate levels of insulin, in September 2016 (FDA, 2016). This device is now used by VA patients.

Summary Dogs played a central role in the understanding of diabetes and the development of insulin therapies for at least 100 years, and it may be surmised that without the unique contributions of dogs, knowledge of the disease's pathobiology would have been delayed along with the development of effective treatments for patients. However, rodents have been the primary species for experimental diabetes research for many years, and the genetic engineering of pigs (described in Chapter 4) has yielded strains with increased similarity to the human disease. Genetically engineered pig models have begun to show promising results for research into pathogenesis, treatment, and transplantation. Going forward, given the current understanding of diabetes pathophysiology and

treatment as well as of the comparative biology of endocrine pancreatic function, there appears to be limited biological justification for the continued use of laboratory dogs, compared with rodents or other non-rodent species.

There are nonphysiological factors, such as growth rate or tractability for specific experimental procedures, that may impinge on species selection and justify the use of laboratory dogs in limited situations. However, given that companion dogs are affected by diabetes, their study offers significant opportunities to advance the understanding of the roles played by genetic, epigenetic, and environmental factors in the development of diabetes. Furthermore, companion dogs with diabetes could directly benefit from research aimed at developing new treatments for the disorder.

Narcolepsy

Narcolepsy is a disruption of normal sleep/wakefulness cycles characterized by daytime "sleep attacks" that affects 1 in 2,000 people in the United States (Mignot, 1997; Zeitzer et al., 2006) and confers a significantly increased risk of injury from motor vehicle accidents (Sakurai, 2013). Narcoleptic patients often suffer from cataplexy, an acute weakening of postural muscles while remaining conscious, as well as hypnagogic hallucinations (Mieda, 2017; Sakurai, 2013). Since the first human studies were performed in the 1960s (Mignot, 2014), investigators have expanded the molecular and clinical understanding of narcolepsy using a variety of model systems, including dogs (Ripley et al., 2001; Tafti et al., 1996), in addition to humans (Sakurai and Mieda, 2011).

Dogs as models of narcolepsy Dogs were first identified as suffering from a form of narcolepsy—and thus of interest as a model for the human disease—in 1972, and this was followed by failed attempts to establish breeding colonies of affected poodles and beagles (Mignot, 2014). It was in the Doberman pinscher that the first genetic transmission of canine narcolepsy was demonstrated in 1976. Canine narcolepsy was well studied from 1977 through 1997, with the majority of research performed on colonies of Dobermans and Labrador retrievers. During that period, numerous sleep studies investigated the clinical manifestations of narcolepsy while genetic studies linked the trait to a single autosomal recessive gene (Baker et al., 1982; Foutz et al., 1979). Dogs also contributed to the understanding of narcolepsy through pharmacologic studies and the exploration of monoaminergic and cholinergic systems as they contributed to sleep states (Karczmar et al., 1970; Mignot, 2014).

As the understanding of narcolepsy advanced, however, the preferred animal model shifted from dogs to rodents. In 1998, two separate research teams independently identified neuropeptides, now known as orexins, that appeared to be deficient in both the dog and human forms of the disease (Hoyer and Jacobson, 2013; Sakurai, 2013). Additional studies revealed that narcolepsy in dogs was caused by a mutation at the level of the orexin receptor, whereas the human disease was due to a deficiency in orexin-producing neurons (reviewed in Hoyer and Jacobson, 2013; Mahoney et al., 2019). With a better understanding of the role of orexins in sleep/wakefulness states, investigators developed knockout mice lacking either orexin-producing neurons (*orexin/ataxin-3*-transgenic) or orexin receptors, thus creating models of either narcolepsy or narcolepsy/cataplexy (Sakurai, 2013). Transgenic rodent models have enabled targeted research into pharmacologic interventions for narcolepsy (Neubauer, 2010; Sakurai, 2013).

VA narcolepsy research using laboratory dogs Narcolepsy affects an estimated 10,000–20,000 veterans (VA, 2018b [protocol no. 10]). Researchers at the VA used laboratory dogs to study narcolepsy in the 1980s, at a time when dogs were the only known model for the disorder. In conjunction with work performed by other research groups, VA scientists were able to identify the parts of the brain responsible for narcolepsy and explore possible treatment approaches (Boehmer et al., 2004).

Subsequent work using murine models and donated tissue from affected humans revealed that the same brain regions involved in narcolepsy are also involved in opioid use disorder, which affects an estimated 131,000 veterans (Baimel et al., 2015; Thannickal et al., 2018). Continuing research into improving treatment for narcolepsy, now performed in mice, has the potential to contribute to the understanding of both narcolepsy and opioid use disorder. The use of dogs to study human narcolepsy at the Los Angeles VA facility, a review of which led some members of the U.S. Congress to question the transparency of VA-supported canine research, as described in Chapter 1, was ended in October 2017 after the principal investigator determined that scientific goals of the study could be met with mice.

Summary Dogs played a significant early role in elucidating the behavior, electrophysiology, and genetics of narcolepsy (Danek et al., 2017; Mignot, 2014; Sakurai, 2013). With improved molecular techniques and the availability of knockout mice that mimic the orexin deficiencies seen in human narcolepsy and narcolepsy/cataplexy, many useful non-dog models are now available to study this debilitating condition (Mignot, 2014; Neubauer, 2010; Sakurai and Mieda, 2011; Tsujino and Sakurai, 2013).

Osteoarthritis and Chronic Pain

Affecting approximately one-third of the U.S. population (more than 100 million people), chronic musculoskeletal pain leads to an economic burden estimated at $600 billion per year, with many currently available analgesics either failing over time or carrying serious risks of side effects (Babatunde et al., 2017; Lascelles et al., 2018). Laboratory dogs have often been used to investigate novel approaches to pain management in concert with rodent or cellular models (Larsen et al., 2009; Pleticha et al., 2015; Wiese et al., 2013; Yaksh et al., 2014), and several recent investigations have turned to companion dogs to study chronic pain and its management (Brown and Agnello, 2013; Carapeba et al., 2016; Cimino Brown, 2017; Hayashida, 2013; Lascelles et al., 2018; Zeira et al., 2018).

An estimated 20 percent of dogs in the United States suffer from OA (Shah et al., 2018). As with people, the most common symptom of OA in dogs is persistent pain, and the spontaneous development of OA is strikingly similar in dogs and humans, causing the same type of damage to bone and cartilage in affected joints (Cimino Brown, 2017). This high disease prevalence and similar pathogenesis, along with the genetic diversity of companion dogs and their shared environments with their human owners, combine to create a relevant model for OA in humans (Cimino Brown, 2017; Lascelles et al., 2018; Zeira et al., 2018). Given the extensive clinical knowledge of OA in dogs and the existence of validated instruments for assessing pain, including the Canine Brief Pain Inventory, the Helsinki Chronic Pain Index, and the Liverpool Osteoarthritis in Dogs owner questionnaire, companion dogs are helping to increase the understanding of how best to treat OA in both dogs and humans (Carapeba et al., 2016; Cimino Brown, 2017; Lascelles et al., 2018; Walton et al., 2013).

Dogs are also informing the treatment of pain from cancer, which they develop at twice the rate of humans (Hayashida, 2013). Investigators are increasingly using companion dogs to assess novel pain management techniques for both arthritis and cancer pain, including the implantation of mesenchymal stem cells; the intra-articular injection of micro-fragmented adipose tissue, hyaluronic acid, or resiniferatoxin; and the intrathecal delivery of substance P-saporin; and they have seen improved outcomes with some of these novel techniques as compared with standard-of-care analgesic treatment (Brown and Agnello, 2013; Carapeba et al., 2016; Iadarola et al., 2018; Shah et al., 2018; Zeira et al., 2018).

There may be a select few circumstances in which a small number of laboratory dogs are needed to provide the homogeneity and controlled circumstances required for a specific investigation focused on translating results to humans (Larsen et al., 2009; Pleticha et al., 2015; Wiese et al., 2013; Yaksh et al., 2014), but an increasing number of investigators are turning to companion dogs to better assess how best to control chronic pain (Carapeba et al., 2016; Cimino Brown, 2017; Hayashida, 2013; Vainio, 2012; Zeira et al., 2018). Researchers have begun to build multi-disciplinary teams focused on translating the results from companion dog studies into effective analgesics for humans, including through a National Institutes of Health (NIH)-supported consortium of veterinary schools and medical schools, discussed in greater detail in Chapter 4 (COHA, 2020). Promising work is also being done in the realm of using companion animals as a treatment adjunct to more traditional analgesic techniques, documenting the pain- and stress-reducing effects on humans of interactions with dogs (Marcus et al., 2012; Pedrosa et al., 2017).

VA osteoarthritis and chronic pain research using laboratory dogs Medications required for effective pain relief or surgical anesthesia can have the side effect of slowing breathing, sometimes to a dangerous degree. As part of an ongoing effort to develop pain management techniques that do not negatively affect respiratory function, VA researchers used laboratory dogs to identify the precise region of the brain responsible for the effects of opioids on breathing (Prkic et al., 2012). The aim of this research is to understand the function of the cells in this region well enough to enable development of alternative medications capable of controlling pain without depressing respiration, to benefit veterans needing long-term pain relief or facing painful surgeries. According to the VA, the meticulous study of individual brain cells that this research required could not have been accomplished in smaller animals (VA, 2018b).

Many veterans experience back pain, often related to their service. In the 2000s the VA used dogs, whose intervertebral discs are similar in size to human discs and undergo disc degeneration, to test a treatment involving the transplantation of autologous cultured cells (Ganey et al., 2003). The results indicated that autologous cell transplant was feasible. Clinical trials based on this research are currently under way (Schol and Sakai, 2019; VA, 2018b).

Summary Companion dogs with OA or cancer offer the opportunity to study and develop treatments for pain that may benefit both dogs and humans. However, when alternatives are not possible, there may be select instances in which a small number of laboratory dogs are needed to provide the homogeneity and controlled circumstances required for a specific investigation focused on translating results to humans.

Experimental Pharmacology and Toxicology

The investigation of pharmacologically active substances is arguably the oldest of the biomedical sciences, originating more than 3,500 years ago. Until the past century pharmaceutical preparations were tested almost exclusively in humans; animal testing occurred infrequently (Kinter and DeGeorge, 2016). The rapid increase in the synthesis of small molecules, which was enabled by advances in organic and analytical chemistry beginning in the late 1800s, was a strong driver in the move toward animal testing. For example, in 1909 Ehrlich and Hata made more than 600 organo-arsenical compounds and tested these in animals to identify "drug 606" (Salvarsan), a treatment for syphilis (Bosch and Rosich, 2008). Ehrlich's early animal experiments, which demonstrated the toxicity of most of the earlier arsenicals, illustrated the strong ties linking chemistry, pharmacology, and toxicology. As more sophisticated animal models became necessary to evaluate efficacy using new technologies (catheters, pressure transducers, electrocardiograms, etc.), an increased reliance

on anesthesia and surgery placed a priority on larger species. Dogs and cats became the most commonly used animal models in pharmacological investigations and remained so through the 1970s.[3]

To obtain a snapshot of dog usage in pharmacological research over the past decade, the committee conducted a literature search for studies published in 2010–2019 that mentioned dogs in the context of pharmacology (see the literature search criteria in Appendix A). This search identified 256 unique publications describing the use of dogs for basic research or the preclinical research and development (R&D) of human therapeutics. The areas of pharmacological investigation included cancer, respiratory, cardiovascular (CV)/renal, bone/dental, endocrine (including diabetes), central nervous system, gastrointestial, musculoskeletal, and experimental surgery. CV/renal was the most frequently cited area (64 citations), followed by bone/dental (40 citations) and central nervous system musculoskeletal (31 citations). Citations associated with product development activities, including pharmacokinetic and toxicity studies, accounted for 85 percent of the total. It bears noting that much product development work involving dogs or any other species is performed to satisfy regulatory requirements and is proprietary, with results seldom published in the public domain, so the published studies may represent a fraction of all product development activities.

VA pharmacology and toxicology research using laboratory dogs The VA is not currently performing primary pharmacology or toxicology research on dogs. However, two projects from the VA's past were noted in communications from the VA to the committee (VA, 2018b). Work performed by VA researchers used dogs to establish the link between smoking and lung cancer, which afflicts veterans at a higher rate than non-veterans (Auerbach et al., 1967). This research has been credited with helping fuel the antismoking movement of the 1970s and saving hundreds of thousands of lives (Burkhart, 1997). In the 1980s, VA research with dogs helped establish a new class of drugs, including ciprofloxacin ("Cipro"), as improved standard treatments for urinary tract infections (UTIs) (Gasser et al., 1987). Dogs were used in order to obtain fluid samples of sufficiently large volume for analysis. This treatment was particularly important for veterans with SCI, who experience a high rate of UTIs.

Currently, the VA only performs pharmacological research on laboratory dogs to support its other studies—specifically, to optimize anesthetic regimens for studying cardiac function. In summary, while laboratory dogs continue to be used for pharmacology and toxicology research, in fields outside of product development they have been largely eclipsed by rodents. The VA currently does not perform primary pharmacological research on laboratory dogs.

Summary The laboratory dog is not currently used as the model of choice for primary pharmacological research unrelated to product development.

Research Areas with Potential Future Companion Dog Use at the VA

This section describes three areas for possible future VA biomedical research involving companion dogs—cancer, infectious disease, and Alzheimer's disease.

Cancer

Cancer is the second leading cause of death in the United States and was responsible for an estimated 599,108 deaths in 2017 (Heron, 2019). The National Cancer Institute (NCI) predicted 1,735,350 new cancer diagnoses in the United States and 609,640 deaths from the disease in 2018 (NCI, n.d.). The incidence and distribution of cancers in veterans, who are predominantly male, are

[3] L. B. Kinter, personal communication, November 6, 2019.

comparable to those in the general U.S. male population (Zullig et al., 2017). The VA strategic plan highlights those areas of medical R&D likely to have the greatest impact on veterans and eligible beneficiaries (Ramoni, 2019; VA, 2019); areas of active interest include environmental exposures during deployment that cause DNA damage leading to cancer as well as the disproportionate impact on veterans of certain lifestyle choices known to cause cancer, such as smoking (VA, 2017). The VA strategic plan also contains a performance goal, anticipating future improvements in the approach to personalized medicine, which would apply clinical genomics to tailor treatment to the needs of the individual. In the area of cancer research, the pursuit of this goal may involve the use of animal models.

Dogs as models in cancer research Historically the laboratory dog played a very limited role in advancing the understanding of cancer biology, particularly when compared to rodents. Rats and mice afforded the benefits of small body size; short life span; genetically defined (and later genetically engineered) inbred strains; extensively studied immune responses; rapid, reproducible tumor induction; and a wide variety of transplantable and inducible tumor models. In contrast, the dog lacked all these characteristics and offered few transplantable tumors, with the exception of transplantable canine glioma (Barker et al., 1993; Salcman et al., 1982) and the transmissible venereal tumor (Bloom et al., 1951; Frampton et al., 2018; Karlson and Mann, 1952; Prier and Brodey, 1963). Efforts at transplanting other spontaneous tumors of the dog required sustained immunosuppression, as in the cases of melanoma (Betton and Owen, 1976) and induced uroepithelial carcinoma (Harzmann et al., 1980). Experimental tumor induction in the dog appears to have little relevance to human cancer and marginal applicability.

Despite the significant limitations of the laboratory dog as an animal model for most cancers, scientists recognized early on that research aimed at understanding spontaneous cancers of the dog might shed light on both human and canine cancer biology (Prier and Brodey, 1963). With the completion of both the human and the dog genome projects (NHGRI, 2019), the genetics underpinning the development of cancers in humans and dogs became available for study and increasingly well understood. These developments have brought the comparative study of cancer in humans and dogs to fruition (NCI CCR, n.d.), and a recent national workshop on this subject drew wide support from veterinary colleges, veterinary and research communities, federal agencies, the pharmaceutical industry, and a variety of patient advocacy and other nonprofit organizations (NASEM, 2015). As noted during this workshop and elsewhere, efforts at translating cancer studies from rodents to humans have encountered significant problems, many of which may be addressed by studying cancer in the companion dog (Gordon and Khanna, 2010; Gordon et al., 2009; Khanna et al., 2009; Paoloni and Khanna, 2007; Paoloni et al., 2014). Advantages of the companion dog include its genetic heterogeneity, large body size, and competent immune system as well as the fact that it has anatomic and physiologic characteristics and genetic/molecular pathways similar to those of humans. Spontaneous cancers of the dog are comparable to the spectrum cancers seen in humans (including histologically) but progress more quickly. Furthermore, dogs and humans have shared the same environment for millennia, potentially shaping similarities in cancer susceptibility through convergent evolution.

Currently, two federal agencies and one nonprofit organization are involved in the coordination and funding of clinical trials and applied cancer research in the companion dog population (ACF, n.d.; NCI CCR, n.d.; VA, 2018b). NCI's Center for Cancer Research's Comparative Oncology Trials Consortium encompasses 20 U.S. veterinary schools and colleges with academic comparative oncology programs that collaborate in cancer research study design, patient recruitment, and implementation (NCI CCR, n.d.). The National Human Genome Research Institute's Dog Genome Project (NHGRI, 2019) has pursued clinical research on bladder cancer, histiocytic sarcoma, and squamous cell carcinoma within its broad portfolio of research on the canine genome. Since the

release of the canine genome sequence in 2005, companion dogs with spontaneous cancers have become a valuable source of biological samples associated with detailed clinical, pathological, and outcome data. In addition to funding new and innovative approaches to cancer therapy, the Animal Cancer Foundation is collaborating with the Canine Comparative Oncology & Genomics Consortium (CCOGC, 2020) to complete tumor genome mapping of all the common canine cancers, to create a resource for researchers investigating canine and human cancer biology.

VA cancer research using companion dogs Melanoma is the most dangerous form of skin cancer and the fifth most commonly diagnosed cancer among veterans, who have a higher rate of melanoma than the general U.S. population (Riemenschneider et al., 2018; VA, 2018b). Research groups in the VA and elsewhere have observed that spontaneous dog melanomas and human melanomas produce analogous antigens, offering hope that the two species could benefit from similar vaccine strategies (Zuleger et al., 2017). VA research, which is performed on companion dogs with spontaneous melanoma, has contributed to the development of experimental vaccines against melanoma for both dogs and humans (VA, 2018b; Zuleger et al., 2017). The VA is continuing to fund this research, which is currently focused on developing new protocols to optimize effectiveness of the vaccines. More recently, VA researchers undertook a clinical trial of a new treatment for bladder cancer in companion dogs. This trial was approved for continuation in March 2018 but was listed as inactive in November 2018 (VA, 2018a).

Summary There is now a well-organized network for scientific collaboration dedicated to advancing the understanding of cancer biology and patient care in humans and companion dogs simultaneously. Research conducted to date through this network, which includes a variety of cancer models with potential relevance to the veteran population, illustrates the validity of this approach. With companion dog patients standing to benefit directly from any improvement in clinical outcome resulting from this research, the adoption of this strategy should significantly mitigate concerns over dog use. In regard to laboratory animal use, while mice remain the predominant model for cancer, the genetically engineered pig—in particular, the Oncopig Cancer Model described in Chapter 4—offers a strong platform for studying cancer and its treatment in a large laboratory animal.

Infectious Disease

Dogs have long contributed to infectious disease research for both human and canine microbial threats, as they are useful for studying pathogens for which dogs are natural hosts (e.g., Lyme disease), reservoir hosts (e.g., leishmaniasis), or models of human disease (Petersen, 2019; Toepp et al., 2019). In their role as companion animals, dogs may transmit infectious organisms to humans as intermediate hosts or pass along antimicrobial-resistant bacteria (Luo et al., 2018; Pomba et al., 2017). One aim of infectious disease research in dogs is therefore to protect humans by preventing zoonotic diseases, such as rabies and leishmaniasis, in dogs (Huang et al., 2015; Toepp et al., 2019).

Laboratory dogs are used to investigate a variety of infectious diseases, with research ranging from basic investigations aimed at understanding pathogenicity (e.g., avian-origin H3N2 canine influenza virus) to vaccine development (e.g., rabies and Lyme disease) (Huang et al., 2015; Lafleur et al., 2010; Loría-Cervera and Andrade-Narváez, 2014; Luo et al., 2018). In recent years, researchers have demonstrated the utility of companion dogs for infectious disease research. Companion dogs are being used to study inflammatory myopathy associated with Leishmania infection, the prevalence of American trypanosomiasis in rural Brazil, and the spread of avian-origin canine influenza virus in the United States (Paciello et al., 2009; Perez et al., 2016; Voorhees et al., 2018). The dog immune response to Leishmania infection, studied in companion animals, has been found

to resemble the human response more closely than that of commonly used mouse strains (Boggiatto et al., 2010; Petersen, 2019; Scorza et al., 2017).

Infectious disease research opportunities using companion or military service dogs While the VA is not currently engaged in canine infectious disease research, this field was included for consideration by the committee due to the presence of U.S. military personnel in areas endemic for visceral leishmaniasis, trypanosomiasis, and additional pathogens not common to the United States (VA, 2020). Leishmaniasis has been described as an emerging infection among deployed U.S. military and civilian workers, with nearly 20 percent of tested soldiers who had been deployed to leishmaniasis-endemic areas of Iraq in 2015–2017 turning up positive for Leishmania infection (as did many deployed dogs) (Mody et al., 2019; Petersen, 2019; Weina et al., 2004). This suggests a possible future role for canine infectious disease research at the VA. Infectious disease research can be designed to benefit military working dogs exposed to unique risk factors as well as humans.

It bears noting that the VA has successfully used dogs to investigate a genetic disorder with relevance to infectious disease. Leukocyte adhesion deficiency (LAD) is an immune deficiency disorder that affects both humans and dogs, leading them to experience life-threatening bacterial and fungal infections and a shortened life expectancy. Studying laboratory dogs with canine leukocyte adhesion deficiency (CLAD), researchers at the VA identified the genetic mutation that causes CLAD and found it to be the same as in some humans with LAD (Kijas et al., 1999). This work was the foundation for an effective gene therapy to treat CLAD in dogs and is being used to develop treatments for LAD in humans (VA, 2018b).

Summary Recent investigations have begun to demonstrate the utility of studying infectious disease in companion dogs, which can benefit both dogs and humans. In some cases, infectious disease research in dogs can include hunt club dogs living in kennels, as opposed to the typical pet dog. With increasing rates of antimicrobial resistance and expanding ranges of potential vectors due to climate change, dogs are likely to continue to play a key role in the study of infectious disease prevention and control, particularly with regard to emerging vector-borne diseases (Pomba et al., 2017; Regier et al., 2016; Uminski et al., 2018).

Alzheimer's Disease

As the sixth leading cause of death in the United States, Alzheimer's disease has long been a topic of interest to VA researchers. Studies performed at the VA have contributed to the understanding, detection, and development of treatments to delay functional decline in Alzheimer's disease; summaries of ongoing research and major accomplishments are available online (VA ORD, n.d.d).

Dogs as models of Alzheimer's disease As the U.S. population ages, with the number of individuals over age 65 expected to nearly double by 2050, the anticipated prevalence of Alzheimer's disease is also increasing, highlighting the urgent need to develop effective therapies and interventions (Alzheimer's Association, 2019).

Alzheimer's disease, the most commonly diagnosed form of dementia in the elderly, is characterized by a variety of destructive neuropathologies, including the deposition of beta-amyloid protein, neuronal loss, and the formation of neurofibrillary tangles, leading to progressive memory loss and cognitive decline (reviewed in Fan et al., 2020). Intriguingly, a similar neuropathology leads to cognitive dysfunction in dogs, pointing to canines as a possible model for the study of Alzheimer's disease (González-Martínez et al., 2011; Head, 2011, 2013; Triani et al., 2018; Vasilevko and Head, 2009; Yu et al., 2011).

Although much animal research to better understand Alzheimer's disease uses transgenic mice or species in which elements of Alzheimer's disease pathology in humans can be introduced (e.g., beta amyloid-infusion rodent models, beta amyloid-expressing nematodes, and even zebrafish) (Jäkel et al., 2017; Van Dam and De Deyn, 2011), investigators have noted several distinctive features of dogs that argue for their special potential in Alzheimer's disease research (Head, 2013; Mazzatenta et al., 2017). Older dogs spontaneously develop neuropathology and cognitive deficits similar to those observed in humans with early Alzheimer's disease, and these deficits can be studied within the context of the dog's unique adaptation for communication with humans and human-like social skills (Mazzatenta et al., 2017). Companion dogs share an environment with their owners that confers similar exposures to pollutants and infectious agents and also share similar activity levels, and thanks to millennia of co-evolution, dogs and humans are able to consume and digest a similar high-starch diet (González-Martínez et al., 2011; Head, 2013). Dogs age rapidly in comparison with people (Kaeberlein et al., 2016), and the key proteins involved in the development of Alzheimer's disease in humans are remarkably similar to those in dogs, with 100 percent homology of beta-amyloid and 98 percent of amyloid precursor protein (González-Martínez et al., 2011). These features make the dog an appealing model for testing candidate Alzheimer's disease interventions (Chapagain et al., 2018; Cotman and Head, 2011). Post-mortem studies of the brains of dogs with cognitive decline, however, do not show the same prevalence of tau-containing neurofibrillary tangles as is seen in humans or other features such as TAR DNA-binding protein 43 inclusions (Smolek et al., 2016).

Extensive research has detailed the behavior and neurobiology of aging dogs (Cotman and Head, 2011; González-Martínez et al., 2011; Head, 2009, 2011; Rusbridge et al., 2018). Efforts to prevent or slow the onset of cognitive decline in dogs have included antioxidant-enriched diets, behavioral enrichments, the drug rapamycin, and additional measures that could potentially serve as models for Alzheimer's disease treatment in humans (Kaeberlein et al., 2016; Mazzatenta et al., 2017; Neumann et al., 2018; Triani et al., 2018). Although there has been some research focused on Alzheimer's disease pathogenesis in laboratory dogs (generally beagles) (Head, 2009), as noted above, more recent studies argue against laboratory dogs as best suited for studies of specific pathologic processes. There remain arguments for testing candidate Alzheimer's disease interventions in companion dogs, whose areas of pathology similar to that observed in humans are further enhanced by the environmental exposures they share with their human owners (Chapagain et al., 2018; González-Martínez et al., 2011; Kaeberlein et al., 2016; Mazzatenta et al., 2017). Dogs therefore offer sufficient age-associated neuropathological overlaps with human Alzheimer's disease to entertain the possibility that a treatment for neurodegeneration in dogs might not only benefit the millions of aging companion dogs but also apply to the treatment of neurodegeneration in humans (González-Martínez et al., 2011; Kaeberlein et al., 2016; Mazzatenta et al., 2017).

Summary Use of aging dogs to study Alzheiemer's disease is a relatively new development, and the VA has not engaged in this research to date. Given recent developments in Alzheimer's disease research, companion and possibly laboratory dogs may play a role in future studies aimed at testing Alzheimer's disease interventions.

Research Areas Previously Investigated by the VA Using Laboratory Dogs

In addition to the research areas described above, the VA submitted to the committee information on six additional research areas in which laboratory dogs had been used. These are discussed in Box 3-1. As described earlier in this chapter, the committee reviewed evidence related to current and recent (since 2016) areas of biomedical research using laboratory dogs at the VA and did not evaluate the necessity of dog use in the areas mentioned in Box 3-1. Nonetheless, it should be

BOX 3-1
Other Research Areas Previously Investigated by the U.S. Department of Veterans Affairs (VA) Using Laboratory Dogs

Within the past decade several studies were conducted at or funded by the VA that addressed the following medical concerns:

- *Organ Transplantation:* Beginning in the late 1950s, VA scientists conducted research in dogs that proved central to enabling the first attempts at liver transplantation into human patients and, ultimately, to the transplantation of other organs as well (Starzl et al., 1963; VA, 2018b). The VA continued to support improvements to the procedures until liver transplantation became established in the late 1970s.
- *Engineering Vascular Scaffolds to Improve Blood Flow for Transplants:* Among injured service members from the Iraq/Afghanistan war, 50–60 percent have injuries to their extremities and 12 percent have injuries to their vasculature, which is necessary for healing. In the 1990s and the 2000s, VA researchers used dogs, whose vascular tissue resembles that of humans, to develop methods of bioengineering vascular scaffolds that could supply the blood flow needed to support tissue transplants and grafts to injured body parts (Pang et al., 2010; VA, 2018b).
- *Managing Gastrointestinal Side Effects of Medical Treatment:* VA scientists conducted research from the 1980s to the 2010s to identify mechanisms responsible for the gastrointestinal side effects, such as vomiting and diarrhea, that are caused by some antibiotics and other medications as well as by radiation exposure (Otterson et al., 2010; VA, 2018b). Some of these mechanisms exist in humans and dogs but not in rodents.
- *Electrical Stimulation of the Gastrointestinal Tract to Treat Obesity:* Obesity and its complications affect 8 million veterans. Many dogs in the United States are obese, and they experience metabolic diseases similar to those in humans. In the 2000s and the 2010s, VA researchers tested mild electrical stimulation of the intestinal tract as a measure for treating obesity in dogs and other animals (Sun et al., 2009; VA, 2018b). This method has subsequently been used in humans to maximize weight loss, and there is evidence that it may be useful for treating bowel dysfunction after a spinal cord injury.
- *Surgical Techniques to Correct Problems That Develop After Hip Replacement:* Roughly 10 percent of people who have hip replacements develop problems that require subsequent surgical revision. VA researchers developed improved techniques for revision surgery in dogs, using bone grafts to rebuild the hip joints before attaching hip muscle to the grafts (Heiner et al., 1994; VA, 2018b). This has led to improved treatments for both veterans and dogs with hip replacements.
- *Understanding Glaucoma:* Roughly 1.5 million veterans have been diagnosed with glaucoma, which is a leading cause of blindness. Dogs with hereditary glaucoma develop a disorder that is almost identical to that in humans. VA research found that the physiological changes of glaucoma are accompanied by retinal inflammation, suggesting a new route for treatments aimed at protecting vision in patients with glaucoma (Grozdanic et al., 2010; VA, 2018b).

noted that several of these studies were active within the past decade and could be revisited by the VA in the future.

NEXT STEPS FOR THE USE OF LABORATORY DOGS IN BIOMEDICAL RESEARCH RELATED TO THE VA'S MISSION

Historically and across disease type, from the understanding and treatment of diabetes to the development of cardiac pacemakers and valve replacement, it would be challenging to find an area of biomedical research that has not benefitted from the involvement of laboratory dogs. The use of dogs in biomedical research peaked in the 1970s, with more than 200,000 dogs per year being used

at USDA-regulated academic and industrial institutions. Since the 1970s, however, as molecular techniques and rodents have gained prominence, some biomedical research has moved away from using laboratory dogs. The focus has shifted toward a variety of alternatives, including rodents, pigs, companion dogs, and even sheep. Today, the majority of dog use in the United States is by companies and private research organizations engaging in applied biomedical research and product development, including testing that is required by regulatory agencies.

Nevertheless, a few areas remain in which laboratory dogs offer the potential for medically important discoveries that cannot currently be obtained elsewhere. These include subsets of CVD research, most notably cardiac rhythm disorders, that depend on anatomical and physiological features that humans share with dogs but not with other laboratory species or on the implantation of devices that rely on restricted growth in addition to some of these other features. They may also include a number of the complex treatments for SCI, particularly cervical injuries resulting in quadriplegia with its many systemic and life-altering effects, that cannot be modeled effectively in other animal or non-animal systems. Even in these limited fields, however, it will be crucial to remain vigilant for non-dog and non-animal alternatives and actively work to promote the development and use of alternatives, as described in Chapter 4.

Given the committee's inability to predict future biomedical research needs, it is not inconceivable that VA research in other fields may require use of laboratory dogs due to unique aspects of their physiology or behavior.

Conclusion 3-1: The laboratory dog is scientifically necessary for only a few areas of current U.S. Department of Veterans Affairs (VA) biomedical research. Based on the request from the VA to review areas of research from 2016 onward, the committee concludes that laboratory dogs currently remain scientifically necessary in these areas of active biomedical research at the VA:

- *mechanistic insights of premature ventricular contraction-induced cardiomyopathy;*
- *autonomic nerve activity and cardiac arrhythmias;*
- *cardiovascular disease requiring functional modeling of the human Purkinje system; and*
- *development and testing of implantable devices to stimulate respiration and cough in spinal cord injury.*

Laboratory dogs are no longer the preferred model for studies of diabetes or narcolepsy, for most imaging studies, or for primary pharmacological research. Responsibility lies with the principal investigator, scientific review committee, and institutional animal care and use committee to know the literature and accurately determine whether the laboratory dog is still the best model for any particular study.

Conclusion 3-2: A potential new approach or treatment may be developed that, for biological reasons, can only be tested in dogs. As yet unknown, new, or reemerging diseases or disorders may not be reproducible in non-dog models and could require the limited use of laboratory dogs to advance their prevention, treatment, or control. Conversely, alternatives may develop in the future that would make the laboratory dog unnecessary.

Conclusion 3-3: The U.S. Department of Veterans Affairs (VA) has an opportunity to expand the study of companion dogs in clinical trials. Companion dogs experience many of the same naturally occurring diseases as humans and stand to benefit from the results

of the research in which they participate. Companion dogs are promising models for a range of disorders, including obesity, diabetes, infectious disease, Alzheimer's disease, osteoarthritis, hereditary glaucoma, cardiomyopathy, thoracic spinal cord injury, and cancer. While companion dog clinical trials can be challenging to conduct due, in part, to the financial and time costs of collecting an appropriate population of companion animals for a particular trial, these studies are possible and deserve priority consideration by VA researchers and leaders.

THE VA'S BIOMEDICAL RESEARCH REVIEW PROCESS

VA-funded biomedical research using laboratory dogs takes place either in VA medical centers or through partnerships with local academic centers (e.g., the St. Louis VA Medical Center no longer has an animal facility on the premises but partners with Washington University and St. Louis University to conduct animal research). VA funds support VA clinician–researchers, who initiate research projects based on the health care needs of veterans. Each laboratory dog research protocol undergoes several stages of VA review, as illustrated in Figure 3-2.

For VA-funded research, the investigator submits a proposal using the same technology infrastructure that is employed by NIH (Grants.gov, n.d.). The proposal is reviewed by a panel of experts who are recruited by the Office of Research and Development of the relevant VA service (primary review) and who score the proposal for scientific merit (see Figure 3-2, bottom left). Completed reviews are sent to the director of the service, who makes the final funding decision. Each facility then carries out a process of regulatory approval. This includes review by a local R&D committee and its subcommittees, which include the institutional animal care and use committee (IACUC) for animal research, the institutional review board for human research, and other committees, depending on the exact requirements of the study. For research with dogs, a secondary veterinary review is carried out by the Office of the Chief Veterinary Medical Officer. The Chief Research and Development Officer; the VA National Center for Ethics in Health Care; the Deputy Undersecretary for Discovery, Education, and Affiliate Networks; and the Undersecretary for Health also review the project before it is submitted to the Secretary of the VA for final approval before the work may begin. The secondary veterinary review was instituted in the 1970s for all VA-funded research involving animals and has been required for all proposed research involving dogs at the VA, regardless of funding source, since 2017 (VHA ORD, 2017). Only after all the regulatory requirements are met is the research permitted to begin. For canine research to be supported by non-VA sources, the same secondary review process applies, with the only difference being that the external funding source, and not the VA, is responsible for the primary review for scientific merit (Bever, 2019). Recent legislative developments related to oversight of VA biomedical research using laboratory dogs are discussed in Chapter 1 and later in this chapter.

Assessment of the VA's Current Review and Oversight Practices

The committee was charged with recommending new or revised scientific parameters for how and when to use dogs for biomedical research at the VA. In order to gain an understanding of the VA's current review and oversight practices, the committee considered the following information sources from the VA:

- Fourteen animal component of research protocols (ACORPs) for VA-funded research projects involving laboratory dogs that were approved or active as of June 1, 2017 (VHA ORD, 2017);

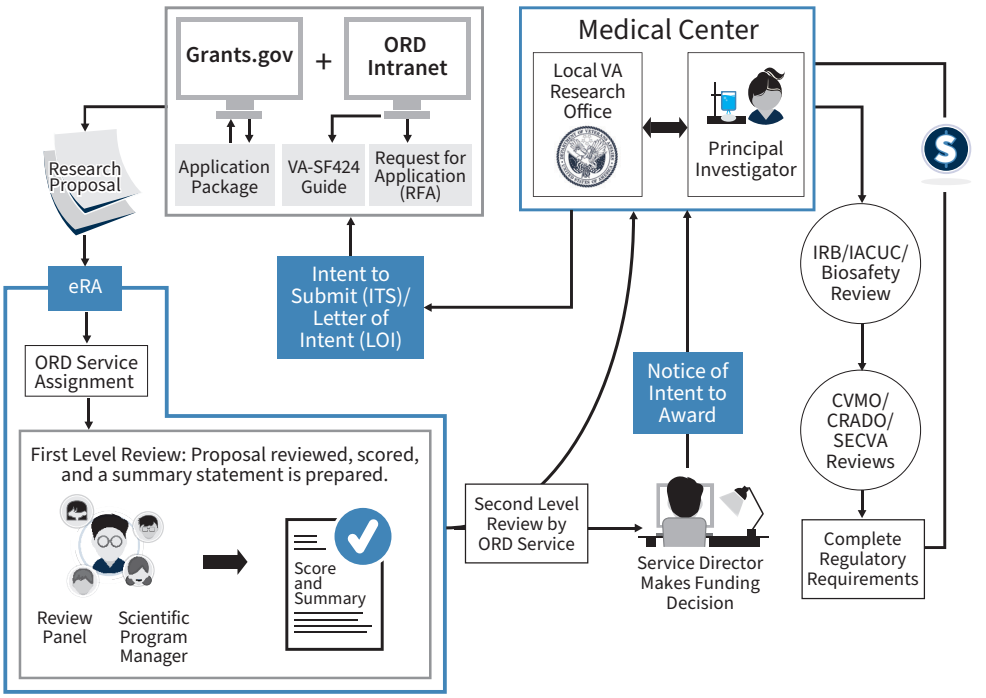

FIGURE 3-2 The U.S. Department of Veterans Affairs' (VA's) intramural canine research review process.
NOTE: CRADO = Chief Research and Development Officer; CVMO = Chief Veterinary Medical Officer; eRA= electronic Research Administration; IACUC = institutional animal care and use committee; IRB = institutional review board; ORD = Office of Research and Development; SECVA = Secretary of Veterans Affairs; VA-SF424 Guide = guide for preparing and submitting VA-ORD applications.
SOURCE: Bever, 2019.

- Four research plans or original grant applications submitted by researchers to receive funding;
- Discussion with VA officials during public meetings to understand the VA research award and review process; and
- Two site visits (Richmond, Virginia, and St. Louis, Missouri) to observe animal laboratories with dogs in VA-funded projects and hold discussions with researchers and IACUC members. Committee members participating in the Richmond site visit on August 20, 2019, were Chris Newcomer and David Powell. Committee members participating in the St. Louis site visit on November 14, 2019, were Chris Green, Nancy Marks, and David Powell. Site visit notes and observations from each site visit were shared and discussed with the full committee.

In considering this information, the committee gained insights into the written materials required from researchers seeking to justify the use of laboratory dogs in a biomedical research study. Site visits and discussions with VA officials provided context and clarification. The VA and its investigators satisfied all requests for information. Nonetheless, the committee's work did not include an organizational analysis of the VA's large, complex research organization and the culture that accompanies it. In the context of launching a biomedical research study with laboratory dogs there are many conversations, decisions, revisions, and requests that do not appear on paper or in the limited discussions that took place between the committee and VA officials and investigators.

Inadequacies in Justifying the Use of Laboratory Dogs in VA Protocols

The majority of the committee's analysis of justifications for using dogs in VA studies relied on the 14 ACORPs for approved or active protocols provided by the VA. Among the 14 protocols, the 2 most common, often jointly occurring, justifications for dog use were (1) their large size and—sometimes—anatomical and physiological similarity with humans and (2) the existence of historical data or the researcher's previous experience with dogs. The quality and extent of justification for using dogs varied across protocols. In some ACORPs the rationale for exclusion of other large animal species, such as sheep or pigs, was their lack of suitable properties (e.g., differences between human and pig Purkinje systems). Some ACORPs only provided a rationale for excluding a single species, and some failed to provide any explanation as to why other large animals were not viable. Most protocols lacked a robust description of a serious attempt to exclude other species or explore alternatives to the laboratory dog.

Inadequacies in Justifying the Number of Laboratory Dogs Used

Among those ACORPs that contained a rationale for the number of dogs used, the committee found the sample size justification to have varying levels of adequacy. Several ACORPs did not have a clear explanation, or any explanation, for the numbers of dogs used, but this reflected the nature of the study (i.e., a pilot study or technique development study that did not require statistical analysis).

Inadequacies in Justifying the Burden and Care of Dogs in VA Protocols

Justifications for the burden and care of dogs ranged from acceptable to marginal. Of note among the protocols reviewed by the committee are two that involved multiple surgeries (two survival surgeries and a terminal procedure) and multiple sedations/anesthesia. The committee was concerned with the performance of multiple surgeries on few individual dogs (to create models of malignant arrhythmias) before an assessment of the effect of sedation and anesthesia on the dog was conducted; the committee felt that these protocols would have benefited from consultation with veterinary specialists (i.e., cardiologists, anesthesiologists, behaviorists). One protocol employed a mix of anesthetizing agents used in modern canine and human anesthesia but lacked any explanation of why these agents were chosen.

Inadequacies in Justifying the Relevance to Veterans' Health

Half of the ACORPs provided an explanation of the relevance of the study to veterans' health, with some ACORPS presenting stronger evidence than others. Some ACORPs explained the relevance of the study to humans in general or to the scientific community but not to veterans specifically.

As concluded in Chapter 2, based on documentation provided by the VA and other organizations as well as on two site visits by subgroups of the committee, the committee finds that the VA's biomedical research programs involving laboratory dogs appear to satisfy applicable laws and regulations surrounding animal research. The primary form of documentation provided to the committee was the ACORP associated with VA protocols using laboratory dogs. The ACORP satisfies legal requirements but, as the conclusions below indicate, the committee believes the VA's review and oversight process could be improved by embracing the spirit of applicable laws and ethical principles.

Conclusion 3-4: The committee was not able to fully evaluate the U.S. Department of Veterans Affairs' (VA's) scientific review process for animal research protocols based on the documents provided by the VA, but the committee's analysis of the animal component of research protocol (ACORP) forms revealed deficiencies in the justification for using dogs instead of other species and for the number of dogs used. The ACORP analysis also revealed instances where the investigators did not adequately explain the relevance of the study to veterans' health.

Conclusion 3-5: Principal investigators frequently cited previous experience with and historical data in dog models as primary justifications for using laboratory dogs. These justifications are insufficient alone and constitute a form of circular reasoning that perpetuates the use of laboratory dogs without adequate examination of alternatives.

Conclusion 3-6: The committee notes that certain protocols would have benefited from consultation with veterinary specialists (cardiologists, anesthesiologists, and animal behaviorists) to address animal welfare issues stemming from the performance of multiple surgeries and multiple sedations or anesthesia on individual dogs and to inform the choice of anesthetizing agents.

OPERATIONALIZING "NECESSARY" AREAS OF AGREEMENT AND DISAGREEMENT WITHIN THE COMMITTEE

The committee offers the following recommendation for determining when it is scientifically necessary to use laboratory dogs in biomedical research funded by or conducted at the VA.

Recommendation 1: Adopt an expanded set of criteria for determining when it is scientifically necessary to use laboratory dogs in biomedical research funded by or conducted at the U.S. Department of Veterans Affairs (VA).

In order to conduct biomedical research that will lead to meaningful outcomes to support improved health of veterans, the VA should adopt an expanded set of criteria for determining if the use of laboratory dogs is scientifically necessary:[4]

1. **The scientific question and the knowledge anticipated will advance understanding or medical practices related to veterans' health;**
2. **Based on unique physiological and other characteristics, there is no alternative to the laboratory dog that will yield scientifically valid results that meet proposed study objectives;**
3. **The anticipated harms experienced by the laboratory dog are outweighed by the potential benefits for veterans; and**
4. **Both the scientific review committee and institutional animal care and use committee have provided written statements attesting that the laboratory dog is the only species that can yield scientifically valid results.**

After reaching agreement on Conclusion 3-1 and Recommendation 1, the committee found itself at an impasse. Ten committee members,[5] a majority, believed that according to the Statement of Task, their job was not done and that a second recommendation linked to Recommendation

[4] Text was modified after the release of the prepublication report to the sponsor to clarify that some of the criteria in Recommendation 1 are not new to the VA. The committee intends for the criteria, old and new, to be applied as a complete set.

[5] W. Ron DeHaven (*Vice Chair*), Joan Hendricks, Jonathan Kimmelman, Lewis Kinter, Nancy Marks, Christian Newcomer, William Potter, David Powell, Margaret Riley, and Rodney White.

1 was warranted, while five committee members[6] were equally convinced that this second recommendation would not be in keeping with the Statement of Task and thus should not be included in the report. The differing opinions of the two groups turn on the meaning of three specific sentences in the Statement of Task—and, in particular, on the meaning of one word that appears multiple times in those sentences.

The crucial word is "necessary," and the portion of the Statement of Task that contains the three sentences reads as follows (with emphasis added):

> Specifically, the committee will write a report to address the following:
>
> (1) Explore recent past, current, and anticipated research questions directly related to the VA's mission to determine if dogs [rather than non-rodent (excluding non-human primates) or rodent species or non-animal alternatives] are or will continue to be *necessary* for relevant basic and translational research. The committee will:
> (a) Make a determination as to whether dogs are or will continue to be *necessary* for any type of biomedical research directly related to the VA's mission. If it is determined that they are *necessary*, describe the unique physiological and other characteristics of dogs that currently make it the *necessary* animal model for use in these types of research.

In reading this portion of the Statement of Task, the majority and minority groups interpret the word "necessary" in different ways and, as a result, end up with differing opinions as to whether the report should include the recommendation in question. While there are other issues that the two groups do not agree on, the crux of their disagreement can be traced to that one word.

To understand the disagreement, some background is useful. Throughout the study process, the committee debated at great length how scientific, legal, ethical, and social considerations factor into the determination of the laboratory dog's necessity in VA biomedical research. In March 2018, prior to the VA's request that the National Academies of Sciences, Engineering, and Medicine undertake this study, the federal government enacted new restrictions on the VA's use of laboratory dogs, mandating that no federal funds "may be used to conduct research using canines unless: the scientific objectives of the study can only be met by research with canines."[7] Section 254 of the Consolidated Appropriations Act of 2018 further required the Secretary of the VA to "directly approve" any such studies, and to submit to the U.S. Congress within 180 days "a detailed report outlining under what circumstances canine research may be needed if there are no other alternatives."[8]

In December 2019, as the committee neared the end of its deliberative process, the Further Consolidated Appropriations Act of 2020[9] was enacted into law. This legislation reiterated the language from the 2018 Act and expanded it to include cats and non-human primates.[10] The 2020 Act also added a new requirement that such scientific objectives must be "directly related to an illness or injury that is combat-related."[11] Furthermore, the 2020 legislation now requires the Secretary of the VA to submit a report to the U.S. Congress for any such approved research "not later than 30 days before the commencement of such research."[12] That report must describe the nature of

[6] Rhonda Cornum (*Chair*), Donna Arnett, Warren Casey, Chris Green, and Sarah Lathrop.

[7] Consolidated Appropriations Act of 2018, Sec. 254, p. 825 (U.S. Congress, 2018).

[8] Id.

[9] H.R. 1865—Further Consolidated Appropriations Act of 2020. Division F Title II Section 249. Available at https://www.congress.gov/bill/116th-congress/house-bill/1865 (accessed March 5, 2020).

[10] Further Consolidated Appropriations Act of 2020, Sec. 249(a)(b), pp. 665–666 (U.S. Congress, 2019).

[11] Id., p. 666.

[12] Id.

the research and include "the justification for the determination of the Secretary that the scientific objectives of such research could only be met using canines, felines, or non-human primates."[13]

In considering the Statement of Task in the context of this legislation, the members of the majority and minority disagreed about the scientific and ethical implications of this legislation and about its relevance to the committee's recommendations to the VA.

As noted, all members of the committee agreed on Recommendation 1, which addresses the Statement of Task—at least partially—by laying out a set of scientific criteria that provides the VA with a framework for deciding when it is scientifically necessary to use laboratory dogs in a proposed protocol. All of the committee members are satisfied that Recommendation 1 provides a framework for *scientific* grounds for determining whether the laboratory dog should be used. The issue that separates them is whether addressing the scientific grounds for using dogs in research is sufficient to satisfy the Statement of Task.

The five committee members in the minority assert that it is indeed sufficient, and they believe that the inquiry should end there. Referring to the definition of "necessary" from the *Oxford English Dictionary*—"required to be achieved" or "essential"—they assert that determining the use of dogs to be necessary in a line of VA research is equivalent to finding that the laboratory dog is the only animal model that can satisfy an important study objective. In short, they believe that the word "necessary" in the Statement of Task should be read as "scientifically necessary" and, in particular, that it was the VA's intention in its Statement of Task to ask only about scientific necessity. Thus, because Recommendation 1 addresses that aspect of the Statement of Task, the minority concludes that no further recommendations are necessary or warranted.

By contrast, the 10 committee members in the majority believe that the discussion of when the laboratory dog is "necessary" should not end with scientific or technical considerations related to the unique physiological and other characteristics of dogs and that parameters for guiding how and when to use laboratory dogs must include ethical, legal, and animal welfare concepts as well. In making this argument, they refer to another part of the Statement of Task, which specifically asks the committee to address other issues than the scientific ones:

> (2) Identify ethical considerations, regulatory requirements, and currently accepted standards for the care, use and welfare of dogs in biomedical research, and make recommendations to enhance their well-being while achieving the research objectives.

If policy making on dog research is to be ethical, the ten members of the majority believe, a broader conceptualization of "necessary" than just "scientifically necessary" is required, and specifically they believe that if policy on dog research is to be ethical it must also take into consideration the implications for members of other species that might be substituted for dogs. In particular, the majority argues that while the *Oxford English Dictionary* provides a concise definition of "necessity," that definition lacks sufficient conceptual or analytical clarity to guide action in this case. Instead they hold that the concept of "necessity" entails specifying at least two factors—the goals invoked and the conditions attached to such goals. The goal of the VA is to restore or improve the health and well-being of veterans, which is done in part by advancing scientific knowledge and the understanding of how diseases (e.g., SCI) affect veterans. Science is not the end goal, but rather science is the means of advancing the goals embedded in the mission of the VA. For research using laboratory dogs to be "necessary," the research must not only advance a set of goals but do so under a set of conditions that include factors like time efficiency, law, and ethics. As described in Chapter 2, any policy that restricts research on dogs may transfer the burden of that research to other large animal models (e.g., pigs and sheep), and the alternative species could potentially experience

[13] Id.

greater harm than laboratory dogs, a scenario that could be considered unethical by some and a violation of the Three Rs—a set of well-established ethical principles that have also been embedded in many regulatory documents guiding the ethical use of animals in research.

The 10 committee members contend that there are no clear moral grounds for arguing that dogs have a "higher" moral status than other large animal species like pigs. Societal preferences, without additional ethical principles, do not confer greater moral value. Therefore, a rule that requires that scientists be more permissive about research on pigs than dogs or similar large animal species is not ethical. What matters most in selecting a particular species, from an ethical standpoint, is that (1) the biological characteristics of the non-human animal indicate that it will provide valuable knowledge in the context of the proposed research, and (2) the harm experienced by the animals is minimized. Some animal research protocols may be less harmful when conducted in dogs than in other species like pigs. Others might be the reverse. The majority of the committee believes these ethical considerations regarding minimizing the harm experienced by animals are part of evaluating scientific necessity.

In support of this contention the majority point to the 2011 Institute of Medicine report on chimpanzees, where the committee found itself in a similar position and took a very similar step. In particular, that committee wrote:

> Neither the cost of using chimpanzees in research nor the ethical implications of that use were specifically in the committee's charge. Rather, the committee was asked for its advice on the scientific necessity of the chimpanzee model for biomedical and behavioral research. The committee agrees that cost should not be a consideration. However, the committee feels strongly that any assessment of the necessity for using chimpanzees as an animal model in research raises ethical issues, and any analysis of necessity must take these ethical issues into account. (IOM, 2011, p. 2)

The committee majority also point to similar ethical considerations being described in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*) (NRC, 2011), which is an internationally accepted primary reference on animal care and use whose use is required in the United States by the U.S. Public Health Service (PHS) Policy. Many institutions, including the VA, are legally obligated to follow PHS Policy and therefore the recommendations in the *Guide*.

In light of such ethical considerations, the majority believes that where multiple species, including the laboratory dog, can be adequately used to answer the scientific question, the species that will incur the fewest burdens should be selected and that such considerations must play a role in determining when a proposed protocol is "necessary."

The committee majority recognizes that this ethical consideration may at times be in conflict with the current law. Specifically, if the dog is the non-primate laboratory species that would incur the fewest burdens in a proposed protocol, ethical considerations would require its use, but that would be prohibited by law. In this case—or in any situation where the animal that would incur the fewest burdens cannot be selected, because of legal or funding restrictions or any other reason—the majority believes that the VA cannot ethically proceed and should consider forgoing the research. In some situations, this limitation might encourage researchers to find an alternative method or combination of methods that would accomplish the goals of the study and reduce harms to an acceptable level. It may require that the research be done by a different entity. It is also possible that if the current legal restrictions on the VA's dog research program are deemed to be hampering the advancement of science, research, and treatments for veterans, lawmakers will choose to remove or alter the current legal restrictions.

With this rationale the 10 committee members in the majority offer the following recommendation:

Recommendation 2: Adopt an expanded set of criteria for determining when to use laboratory dogs in U.S. Department of Veterans Affairs' (VA's) biomedical research when the dog is not scientifically necessary.[14,15]

In order to conduct biomedical research that will lead to meaningful outcomes to support improved health of veterans, the following criteria should be met before approving the use of laboratory dogs when other animal models are also scientifically appropriate:

1. **The scientific question and the knowledge expected to be gained will advance understanding or medical practices related to veterans' health;**
2. **The research objective cannot be adequately addressed using new approach methodologies or ethically using human subjects or companion animals;**
3. **Where multiple species [excluding non-human primates],[16] including the laboratory dog, can be used to adequately answer the scientific question, the non-primate species that will incur the fewest burdens should be selected. If the species that will incur the fewest burdens cannot be selected for any reason, including legal and/or funding restrictions (e.g., the laboratory dog), the VA cannot ethically proceed and should consider forgoing the research; and**
4. **The expected harms experienced by the selected animals are sufficiently outweighed by the expected benefits for veterans. Both the institutional animal care and use committee and the VA's central office ethics review should concur in this assessment.**

The five member committee minority dissents from this recommendation because they believe the recommendation strays beyond the Statement of Task. Where the majority grounds its arguments for the recommendations in ethical considerations, the minority points to the ethical, legal, regulatory, and institutional context in which the Statement of Task was prepared and argues that the VA clearly intended the committee to address only the scientific necessity of using dogs in research and that ethics-based recommendations are outside the scope of what the committee was asked to do.

To support its position, the minority offers several observations. First, turning to the Statement of Task, the minority points to the sentence "If it is determined that they [dogs] are necessary, describe the unique physiological and other characteristics of dogs that currently make it the necessary animal model for use in these types of research." The implicit message in this sentence, the minority argue, is that the VA wanted the committee to focus on objective scientific criteria, such as physiological characteristics, in the determination of whether dogs are necessary in particular research projects. A second sentence in the Statement of Task asks the committee to "Provide recommendations for any new or revised scientific parameters to guide how and when to use dogs

[14] Five committee members (Rhonda Cornum [*Chair*], Donna Arnett, Warren Casey, Chris Green, and Sarah Lathrop) dissent to Recommendation 2. The dissenters acknowledged the English dictionary definition of "necessary" ("required to be achieved, or essential") as outlined in the Statement of Task to recommend to the VA when the laboratory dog was necessary in biomedical research (i.e., the dog is the only model that will yield scientific results directly related to veterans' health). This is exactly what federal law currently directs. As the Statement of Task did not request an evaluation of other animal models, the dissenters conclude the majority's Recommendation 2 strays beyond the Statement of Task. Additionally, the five committee members argue that a broader ethical framework that is responsive to the public's perception of animal research be considered, especially given that research conducted by the VA is publicly funded.

[15] Text was modified after the release of the prepublication report to the sponsor to clarify that some of the criteria in Recommendation 2 are not new to the VA. The committee intends for the criteria, old and new, to be applied as a complete set.

[16] Non-human primates were excluded from the committee's consideration according to the Statement of Task provided by the VA.

for biomedical research rather than non-rodent (excluding non-human primates) or rodent species or non-animal alternatives." Again, only scientific parameters, not ethical ones, are mentioned. Thus, the minority believes that the VA's intention in its Statement of Task was that the committee address only the scientific necessity of using dogs in research.The minority also believes that the ethical considersations (#2 in the Statement of Task) were specific to the dog (rather than to a comparative ethical analysis).

More broadly, the minority contends that Recommendation 2 is in conflict with both existing VA policy and existing federal legislation. For example, during the public meeting held in Washington, DC, on February 14, 2019, the VA representative confirmed that the definition of "necessary" used by the VA holds that the VA should only continue using dogs in biomedical research when there is no other alternative that will yield scientifically valid results that meet proposed study objectives of importance to veterans (i.e., the definition it uses of "necessary" is essentially "scientifically necessary"). Furthermore, the federal appropriations legislation that places restrictions on the use of dogs in VA-funded research uses the same definition of necessity. Beyond that Recommendation 2 contradicts accepted policies of various other federal agencies, such as NIH, many of which treat some animals, such as the chimpanzee, differently from others, and the Animal Welfare Act privileges any "dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal" over every other type of sentient species, including rats and mice, birds used for research, and farmed animals, such as horses, livestock, and poultry.[17]

Thus, the minority members believe that while it is certainly important to grapple with the broad ethical issues surrounding animal research, this report is not the proper venue, as it was intended to address scientific questions and ethical and regulatory positions taken by the VA and the federal government specific to the laboratory dog. Furthermore, the minority contends that if a full ethical analysis of using various species of animals in biomedical research were to be undertaken, it would require a deeper analysis than this committee was equipped to perform and likely would require convening a committee that was not composed predominantly of scientists. If the dissenting minority believed a comparative ethical analysis had been requested in the Statement of Task, the minority would have proposed the use of a broader ethical framework that acknowledges the rapid changes in science (e.g., the sophistication of non-animal models), is inclusive of societal values (particularly when the research is publicly funded), is ethically defensible, and is ultimately useful to researchers.[18] Regardless, the minority members still conclude that such comparative ethical questions remain outside the scope of the committee's Statement of Task. For these reasons they dissent from Recommendation 2.

To sum up, the disagreement between the majority and minority over Recommendation 2 is essentially a disagreement about whether that recommendation comports with the Statement of Task. The majority, taking a broad view of the meaning of "necessary," believes it does. The minority, holding a more restricted view of the meaning of "necessary," believes it does not. The practical effect of that definitional disagreement is that the majority believes that the interests of other laboratory animals than the dog must be taken into consideration when determining the necessity of research on laboratory dogs, while the minority believes that the question the committee was asked dealt not with other research animals but only with laboratory dogs.

[17] 7 U.S. Code § 2132(g). The section goes on to clarify the Animal Welfare Act's (AWA's) protection of dogs by specifically stating that, "With respect to a dog, the term means all dogs including those used for hunting, security, or breeding purposes." And when first proposed in the mid-1960s, the working title of the bill that eventually became the AWA was "The Dog Protection Act."

[18] This minority approach would closely mirror the framework proposed to the committee by animal ethicist Dr. David DeGrazia, Senior Research Fellow, Department of Bioethics, National Institutes of Health; Elton Professor of Philosophy, The George Washington University.

OPPORTUNITIES TO IMPROVE BIOMEDICAL RESEARCH PROTOCOLS AND REVIEW PROCESSES AT THE VA

The committee is concerned that the current culture of justifying biomedical research in laboratory dogs favors the continued use of dogs, given that the previous experience of principal investigators and technical support staff, along with the availability of extensive historical data for dogs, are sometimes the sole justifications provided for the continued use of laboratory dogs in research. The current system has considerable momentum around scientific review committee and IACUC acceptance of the principal investigator's judgment regarding whether laboratory dogs are necessary. In the experience of some committee members, in general, if an investigator states that the harm to laboratory dogs used in a particular study would be less than the harm incurred by pigs or sheep because more pigs or sheep would be required to build the knowledge base to a level comparable to dogs, it is possible that scientific review committees and IACUCs will accept that logic. Perpetuating this reasoning favors the continued use of laboratory dogs in biomedical research, at the VA and elsewhere.

The committee could not fully evaluate or understand the institutional momentum at the VA for or against this reasoning for the continued use of laboratory dogs. Rather, this concern is based on review of ACORPs and the opinion and experience of some on the committee. The VA Chief Research and Development Officer, Rachel Ramoni, told the committee that the VA was committed to moving away from sensitive species, including dogs, even if that meant an additional investment of time and money (Ramoni, 2019). Indeed, the committee learned that a pilot study in pigs was under way at a VA center to potentially replace the use of laboratory dogs. Also during the course of this study, several VA protocols planned in laboratory dogs were switched to mice, pigs, or humans.

Justifying the use of laboratory dogs at the VA today requires significant paperwork and sign-offs but, in the committee's judgment, does not always require a thorough examination of alternatives nor an accurate accounting of harm and burden to study animals. For example, evidence from the ACORPs reviewed by the committee suggests that literature searches for relevant alternatives are of poor quality and seem to be primarily box-checking exercises (i.e., the decision to use laboratory dogs having been made prior to the literature search for alternatives). Also, some ACORPs lacked a written descripton of the perceived harm to laboratory dogs. A few ACORPs also seemed to misapply the Three Rs by prioritizing reduction in the number of dogs used over refinement.

Tracking Impact of Laboratory Dog Research Through Prospective Registration: A Strategy for Improving Quality and Reducing Animal Use

In recent years, many commentators have encouraged the prospective registration of preclinical studies, particularly those designed to test disease interventions and toxicology in animals (Anderson and Kimmelman, 2012; Heinl et al., 2020). This process would require that full experimental design and results from preclinical studies be deposited in publicly accessible databases regardless of the studies' outcome. Prospective registration can improve the quality and refinement of animal studies (Wieschowski et al., 2016). Full disclosure of experimental procedures would be likely to increase the reproducibility of research, while the disclosure of results would reduce animal use by avoiding unnecessary duplication of studies, because failed experiments—which rarely see publication in the scientific literature—would not be repeated.

These calls echo those that motivated the establishment of clinical trial registries, such as clinicaltrials.gov, for research involving human beings (De Angelis et al., 2004). Clinical trial registries were established following several episodes in which information adverse to particular pharmaceutical products was found to have been withheld from publication.

While there are currently no requirements in the United States for the preregistration of preclinical trials, establishing this as standard procedure for studies that use laboratory dogs could both enhance the knowledge gained from this research and improve its harm–benefit ratio. Though no gold-standard registration portal has yet emerged, several options exist; these include the Open Science Framework (osf.io) and two international registries, preclinicaltrials.eu and animalstudyregistry.org (Bert et al., 2019).

Recommendation 3: Improve biomedical research protocols and review processes, and track the impact of research.

The U.S. Department of Veterans Affairs (VA) should enhance its scientific and ethical review process so that it better integrates the assessment of harm and burden with assessments of value and impact associated with biomedical research using laboratory dogs. There should be an explicit and strong connection between scientific review and institutional animal care and use committee (IACUC) consideration so that all reviewers understand the study objectives, harm–benefit assessment, and anticipated value and impact of the study on human health. The VA should focus efforts on improving the following areas:

- **Protocol Development. Specifically, the VA should implement measures to ensure that:**
  - **The principal investigator starts prior to submission to a funding agency to:**
    - **Develop the biomedical research question and fully describe its value to the VA's mission, veterans, and the nation;**
    - **Engage with an independent literature research group to ensure thorough and transparent evaluation of possible new approach methodology (NAM) alternatives (discussed in Chapter 4);**
    - **Consult with the attending veterinarian to determine whether the requisite veterinary expertise is present in the VA. If additional expertise is needed, the VA's principal investigator should be supported in engaging with veterinary specialists outside the VA to develop protocols and refine procedures necessary to meet study objectives. Examples include newer imaging techniques to measure anatomical and functional parameters of tissues; minimally invasive surgical and interventional radiographic techniques for device placement; and contemporary pain assessment and relief, including current measures of inappetence, weight loss, and other clinical parameters;**
    - **Engage with independent statisticians to ensure appropriate study design and statistical power analysis; and**
    - **Submit the research protocol to funding agency (the VA or other) and IACUC simultaneously.**
- **Protocol Development and Review Processes. Specifically, the VA should:**
  - **Emphasize the replacement of laboratory dogs and the refinement of procedures and techniques over a reduction in animal numbers in order to reduce the burden on individual dogs, even if more animals (including alternative species) will be used;**
  - **Improve literature searches for alternatives to laboratory dogs. The VA should fund an independent party to conduct literature searches designed to yield objective, independent analyses of the need to perform proposed**

research in laboratory dogs versus alternative animal models, NAMs, humans, or human tissues; and

- **Engage with board-certified and other experts in canine medicine and research to review research goals and ensure optimal study design, including estimates of the sample size needed to ensure adequate statistical power. Consider spontaneous clinical conditions of relevance and the possibility of clinical trials in companion dogs to complement or replace laboratory dog studies.**

- **Track Impact of Research. Specifically, the VA should:**
  - **Establish a mechanism for tracking the impact and translation of research using dogs. Such a retrospective reporting mechanism should use objective and state-of-the-art methods (e.g., bibliometrics or citation in regulatory documents and patents) to track the relationship between dog experiments and translated interventions for veterans. Such performance assessment should be required to establish and, if need be, correct risk–benefit and welfare assessments used in the authorization of research.**
  - **Take steps to encourage the prospective registration of all studies involving laboratory dogs.**

Recommendations 1, 2, and 3, if adopted and enforced, would become part of the culture and process of scientific and ethical review at the VA. Recommendations 1 and 2 would create the expectation that principal investigators consider, early in the study proposal process, all possible alternatives (non-animal or animal) and the relative harm that the proposed study would bring to the candidate subjects. Scientific review committees and IACUCs would be conducting simultaneous reviews of the analysis of harm and benefit, such that all three parties—principal investigator, scientific review committee, and IACUC—would develop an agreed-upon understanding of "scientific necessity," reconcile any differences of perspective related to the proposed study, and generally pool accountability for decisions related to the use of laboratory dogs.

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NOTE: An asterisk before the first author's name denotes that the publication was based on research conducted at or funded by the VA. For such references, details about the VA connection are appended after the reference.

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4

Alternatives to the Use of Laboratory Dogs

The preceding chapters evaluated the use of laboratory dogs in biomedical research in fields relevant to the mission of the U.S. Department of Veterans Affairs (VA). This chapter explores the landscape of alternatives development in areas of interest to the VA where laboratory dogs are currently used or were used in the recent past—cardiovascular disease (CVD), spinal cord injury (SCI), cancer, and diabetes. Alternatives considered in this chapter include companion dogs, other laboratory species, and non-animal methods.

CURRENT STATUS OF ALTERNATIVES DEVELOPMENT

While laboratory dogs have been used to study a wide range of diseases throughout history, the frequency of their use for research purposes, both in the VA and throughout the United States, has declined over time for a variety of reasons, as described in Chapter 3 and Appendix B. In documents provided by the VA to the committee, justifications for using laboratory dogs in biomedical research largely rely on the availability of historical data and biological similarities between dogs and humans. The committee notes that reliance on historical data and the biological similarities between dogs and humans is not unique to researchers or the scientific review process at the VA—biomedical researchers at institutions throughout the country and world often rely heavily on these factors to justify the use of laboratory dogs or, for that matter, any species. Shifting research to an alternative model (animal or non-animal, including humans) will require successfully addressing those factors that favor the continued use of laboratory dogs.

Four broad categories of alternatives are considered in this chapter. The first is companion dogs volunteered by their owners for clinical trials. The second is laboratory animal models other than the dog, and the third is "new approach methodologies" (NAMs), a diverse array of innovative non-animal approaches. Finally, the fourth category consists of areas where human clinical trials could be used to address research questions currently being studied in laboratory dogs.

As a promising medical discovery is shepherded along the translational pipeline, with testing frequently required in both small (rodent) and large (non-rodent) mammals, it is essential to select

the most scientifically valid approach to study the disease of interest. For animal models, criteria have been proposed to define the ideal model (Schachtschneider et al., 2017a). This model would, for example, mimic a human disease at the molecular and physiological level and display a comparable natural history of the disease, be associated with cell lines for in vitro study, yield reliable and predictable results, provide accurate treatment assessment, and be amenable to imaging.

Although animal models will continue to be part of advancing biomedical research in the near future, they are not always capable of representing the nuances of human pathophysiology and genetic variability critical to efficient translation with impactful clinical outcomes. The desire to improve the translational impact of biomedical research, together with societal pressures to reduce the use of animals for this purpose, has led to significant interest and investment in human-based approaches to biomedical research that do not require the use of animals. These approaches are referred to herein with the broadly descriptive term NAMs, which includes any non-animal technology, methodology, approach, or combination thereof, including in vitro, ex vivo human tissue, computational, and in silico models. While not capable of recapitulating the complexity of intact animal models, NAMs do offer the potential to evaluate specific critical aspects of human biology that are not replicated in animals.

COMPANION DOGS

For multiple reasons, companion dogs (in lieu of laboratory dogs) provide an attractive model in which to study serious medical conditions that affect veterans. In these studies, new diagnostic, preventive, or therapeutic approaches are investigated in dogs with conditions (or genetic predispositions to conditions) of interest. The dogs are "volunteered" by their owners and reside with their owners while under study. The study interventions are carried out in appropriate veterinary clinical settings. Investigators may include licensed veterinary clinicians, Ph.D. biomedical scientists, and physicians focusing on humans, often working as teams. Ethical, legal, and investigative standards comply with federal guidelines for the human owners—if data are collected from them—and also the dogs and the clinical facilities where study interventions are conducted. Veterinarians and animal health businesses have long studied companion dogs as research subjects to enhance the outcomes in the canine patients themselves. Properly controlled and conducted studies also have the potential to provide preclinical data for similar approaches in humans, including veterans. Such use could reduce the use of laboratory dogs while potentially benefiting the companion dogs, proving advantageous on both ethical grounds and in the eyes of the public. As discussed in Chapter 3, companion dogs may also offer certain scientific advantages over highly controlled studies using laboratory dogs. For example, while laboratory animals are generally chosen for their homogeneity, companion dogs display both (well-characterized) genetic heterogeneity and environmental diversity, similar to humans. We note that the genetic and environmental homogeneity of animals bred for laboratory research—notably rodents—can be essential during the early stages of discovery, reducing variables and enabling phenomena to be studied in relative isolation. These studies may be more scientifically valid, easily replicated, and cost-effective as well as more ethically appropriate than initial studies conducted in pet animals. However, evaluations of treatment efficacy of the sort likely to involve dogs may benefit from a more diverse population, and while such studies are more expensive and potentially involve larger group sizes than some studies in laboratory animals, they are certainly less expensive than human clinical trials.

As noted in Chapter 3, many conditions that the VA was investigating prior to the initiation of this study, including cardiac arrhythmia, cardiomyopathy, SCI, cancer, and narcolepsy, are experienced as spontaneous conditions in the companion dog population. Other topics of research at the

VA that are endemic to dogs include infectious diseases, chronic pain, senile dementia, and obesity. Additionally, military working dogs experience physical trauma, and there is general clinical suspicion that psychological trauma may also occur, with dogs going on to develop canine posttraumatic stress disorder (Broach, 2018).

There are limits to the utility of companion dogs as research subjects. One limit is biological and results from the significant differences between humans and dogs with regard to disease prevalence, natural history, and/or pathophysiology. For example, while dogs display cardiac arrhythmias, cardiomyopathy, and heart failure, they do not experience atherosclerosis at anywhere close to the human rate. Diabetes mellitus (DM) occurs at a rate of roughly 0.2 percent in companion dogs, with type 1 more prevalent than type 2 (Banfield Pet Hospital, 2016). Although dogs contract some infectious diseases in common with humans (e.g., rabies, Lyme disease, leishmaniasis) (CFSPH, 2011; Petersen, 2019), the manifestations can be different; for example, the most common serious sequela of Lyme disease in dogs is kidney disease, but neurologic disease is more common in humans. Furthermore, many significant human infectious diseases do not affect dogs.

Although many types of human cancer are seen in companion dogs, the use of companion dog models could be limited by certain factors. Recruiting sufficient numbers of companion dogs with a particular cancer for a randomized controlled trial in a timely manner could be a challenge, for example. In cancer and other disease areas, the variability inherent in companion dog clinical cases may require greater treatment group sizes than if laboratory dogs are used. In companion dogs (depending on the scientific questions that the researcher wants to address), controlling environmental factors, such as outdoor exposure, diet, and environmental stressors, all of which may be confounders, may also be a challenge. The use of companion dogs also limits the ability to collect certain experimental endpoints that can be obtained from laboratory dogs, such as histopathology, gross pathology, and necropsy.

Companion dogs experience natural SCI at a high rate in the thoracic and lumbar regions, but cervical injuries—the most common type in humans and an area of research at the VA—are rarely seen in companion dogs. If a dog with a high spinal injury suffered respiratory paralysis or severe compromise, sustaining it through prolonged ventilator support would not be consistent with humane and ethical practice; this also applies to the long-term study of cervical SCI in laboratory animals. Therefore, companion dogs can be useful for studying SCI and its response to treatment but not for research on quadriplegia or long-term respiratory support.

While studying a disease of interest in companion dogs is more attractive than working on laboratory animals on both ethical and public perception grounds, it requires certain preconditions that have yet to be met for many disorders. First, the disease must have been sufficiently well studied in dogs to establish its relevance for humans. Second, it requires that researchers studying the human condition collaborate with clinician–scientist partners competent in companion dog studies. Both conditions have been met in the fields of cancer and chronic pain, as discussed further below, and there is the potential to satisfy them for other areas of interest to the VA.

In addition to biological and scientific constraints, another factor limiting the expansion of research to companion dogs is administrative. With notable exceptions (such as for cancer and SCI, as discussed previously), there has been little investment in developing the infrastructure needed to enable biomedical researchers to identify those human diseases that are amenable to being studied in dogs and to find high-quality clinical trials with which they might collaborate. For many diseases, even where the science supports use of a companion and a working dog model, there is no existing network for conducting clinical trials in dogs. This is not an insurmountable issue; indeed, a relatively minor investment in administrative infrastructure and network building could yield significant value.

Current State of the Art for Companion Dog Clinical Trials

Veterinary clinical trials have a long history (Boothe and Slater, 1995), and the past decade has seen an increased interest in using companion dogs for these studies (Davies et al., 2017; Dean, 2017; Giuffrida, 2016; Rice et al., 2008). International guidelines for carrying out these trials have been discussed, with a focus on regulatory drug approval studies (Alvarez and Fortsch, 2005), although no international regulations are currently in effect (*Nature*, 2016).

Among the studies performed on companion dogs, the most longstanding and well organized are those investigating treatments for cancer (Paoloni and Khanna, 2008). While juvenile dogs usually die from trauma, congenital disease, and infections, cancer is the leading cause of death in adults (Fleming et al., 2011). Dogs spontaneously develop cancers at rates similar to those in humans, and companion dog models have been successfully used in preclinical trials of cancer therapies (Cornelius, 2018; MacNeill et al., 2018).

The National Cancer Institute (NCI) supports a Comparative Oncology Trials Consortium (COTC) using companion dogs to study cancer (NCI CCR, n.d.a). COTC studies have demonstrated an exemplary level of collaboration between canine-focused and human-focused clinicians and resulted in multiple publications (NCI CCR, n.d.b).

A consortium to investigate spinal cord injuries, the Canine Spinal Cord Injury Consortium, is also well developed, with promising results from a recent trial that tested cell-based therapy in companion dogs with SCI (Jeffery, 2019; Moore et al., 2017) and another that tested intraspinal injection of chondroitinase (Hu et al., 2018; Jeffery, 2019). Studies of novel approaches to relieving pain caused by chronic disease, which is particularly difficult to study humanely in a laboratory setting, have also yielded promising results (Cimino Brown, 2017; Iadarola et al., 2018).

The committee's task included the consideration of future areas of research likely to be relevant to veterans. These future topics may include, for example, injuries sustained in battle. Dogs endure battleground injuries, and a recent article in *Military Medicine* called for the establishment of a military working dog trauma registry to collect these data to improve casualty care (Orman et al., 2018). There is also interest in the diagnosis and treatment of canine PTSD, which, due to the nature of their work, military working dogs are prone to develop (Broach, 2018).[1]

Indeed, the VA is to be recognized for its pioneering research using companion dogs, including a current evaluation of immunocytokine therapy for melanoma (VA ORD, n.d.). If media coverage is any indication, then public interest in companion dog research is high (NCI CCR, n.d.b). Another indicator of such interest occurred on December 19, 2019, when the U.S. Senate unanimously passed Senate Resolution 462, designating January 2020 as "National One Health Awareness Month" to promote collaboration among public, animal, and environmental health scientists (Feinstein, 2019).

Optimization of VA Research to Use Companion Dogs

The VA has an opportunity to become a premier biomedical research entity engaging formally with veterinary expertise, both to enhance the experience of laboratory dogs and to conduct clinical trials in companion dogs, using companion dog studies to replace laboratory dog research wherever possible. Accomplishing this goal will require the following:

1. **Engaging with experts in canine medicine and research to optimize both clinical methods and research goals.**

[1] While clinical criteria have been established by the American College of Veterinary Behaviorists (https://www.cliniciansbrief.com/article/acvb-avsab-capsules [accessed June 16, 2020]), as of this writing, there are no published scientific studies of canine PTSD.

The study of comparative species medicine and biomedical research underpin the fundamental argument that studying non-humans will inform us about humans. Veterinarians dedicate their careers to this work, and the application of veterinary expertise to more than simply assuring humane treatment and regulatory compliance would strengthen the quality of the VA's work. While board-certified veterinary clinical specialists could (and should) ensure that the most modern techniques are applied to all procedures, the VA can aim higher, with the broad evolutionary perspective of "One Health" serving as a guide to new avenues of inquiry.

"One Health" highlights the intellectual and practical benefits of recognizing the linkage between human health and veterinary medicine. To enable a more holistic approach to human, animal, and environmental health, many veterinary colleges have relationships with parallel health colleges. The Association of American Veterinary Medical Colleges (AAVMC) provides leadership and liaison to all veterinary schools accredited by the American Veterinary Medical Association (AVMA) Council on Education, the accrediting body for all colleges and schools of veterinary medicine in the United States. The AAVMC, with its mission to advance the quality of academic veterinary medicine, serves to convene those schools that support improving the quality of research conducted on domestic animals. Additionally, the AAVMC liaises with AVMA's American Board of Veterinary Specialties, providing a link to the mostly highly trained and credentialed specialists.

Of particular relevance to the VA at present is a formal memorandum of understanding among the cardiology colleges, which engages veterinary experts and non-veterinarians involved in biomedical research related to cardiovascular conditions and diseases shared across multiple species. Thus, the AAVMC is positioned to provide expertise to the VA, should it choose to proactively seek veterinary input to develop and refine studies that may benefit the species of animal under study as well as the primary intended beneficiaries, veterans.

2. Collaborating with researchers conducting clinical trials in companion dogs to identify or develop trials to benefit veterans.

Animals in general, and dogs specifically, often experience disorders similar to those being researched by the VA. As a result, studies aimed at improving the understanding, diagnosis, treatment, and prevention of these disorders in dogs can benefit humans as well. There is a need for a standardization of methods to ensure that clinical trials performed on companion dogs produce high-quality results (Boothe and Slater, 1995; Davies et al., 2017; Dean, 2017; Giuffrida, 2016; Rice et al., 2008). Nonetheless, clinical trials have become a focus of academic veterinary centers internationally (Alvarez and Fortsch, 2005), with an increasing commitment to conducting high quality, multi-center trials. Such trials are subject to ethical and legal regulation to safeguard both owners and patients. The most longstanding and well-organized area of interest is cancer, with NCI supporting the COTC, as described earlier (NCI CCR, n.d.a). Several modes of treatment, especially immunological approaches, have moved forward into human clinical use as a result of such trials (Dow, 2020; Gardner et al., 2016; NCI CCR, n.d.b; Prouteau and Andre, 2019; Tarone et al., 2019). As described in Chapter 3, a consortium to investigate SCIs is also well developed (Moore, 2019; Moore et al., 2017).

As described above, studies of novel approaches to pain relief from chronic disease have yielded promising results, providing evidence that results obtained in companion dogs may reliably predict efficacy in humans (Cimino Brown, 2017; Lascelles et al., 2018). The genetic, environmental, and lifestyle variation seen in companion animals may offer a better model of the human condition—and therefore of much human disease—than the highly controlled genetics and environment that are standard for laboratory animals (Lascelles et al., 2018).

3. Participating in efforts to develop a registry connecting human research needs with companion dog clinical trials.

Biomedical researchers and clinicians need to know the status of clinical trials in their field. Most desirable would be a fully funded registry to help researchers in human disease quickly determine whether a relevant companion dog trial exists or could be developed. AVMA has an online clinical trial registry, albeit one focused on linking pet owners and primary care veterinarians with clinical trials (AVMA, n.d.). To fill in the missing link—that is, connecting biomedical researchers focused on human disease with companion dog clinical trials—perhaps the best route would be through an existing consortium of veterinary schools and medical schools supported by the National Center for Advancing Translational Sciences institute of the National Institutes of Health (NIH). This consortium, the Clinical and Translational Science Award One Health Alliance, currently consists of 15 schools and has received funding to enhance its presence and impact. Its website is designed to educate and link researchers who are interested in such studies (COHA, n.d.).

The committee envisions the VA becoming a pioneer among biomedical research institutions in fostering collaborative work to benefit both veterans and canine patients, thereby addressing many of the current ethical difficulties inherent in using laboratory dogs. It is notable that companion dog studies receive enormous positive public interest and, most importantly, have the potential to accelerate the translation of biomedical research to improvements in human health.

Conclusion 4-1: The use of companion dogs in biomedical research aimed at benefiting both dogs and humans is a preferred alternative to the use of laboratory dogs. Companion dogs experience many of the same naturally occurring diseases as humans and stand to benefit from the results of the research in which they participate. Established areas of clinical companion dog research with relevance to preclinical studies in veterans include cancer and (thoracic) spinal cord injury. Other disorders of interest to the U.S. Department of Veterans Affairs likely to benefit from development of a companion dog model include chronic pain, diabetes, cardiovascular disease, and senile dementia, including Alzheimer's disease. The utility of companion dogs may increase if other biomedical research areas wherein their use is scientifically valid could be identified and if there is an infrastructure in place to facilitate the conduct of studies that use companion dogs.

Conclusion 4-2: A significant barrier to conducting clinical studies in companion dogs is a lack of administrative infrastructure to connect U.S. Department of Veterans Affairs (VA) investigators to the veterinary researchers who conduct such trials. The regulatory infrastructure to address ethical and legal issues for clinical trials in dogs is already established, but the mechanism for using these studies to supplement, complement, or accelerate collaborations with investigators who are interested in conducting human clinical trials does not exist. With validation of the utility and relevance of the naturally occurring canine disease or disorder for the study of the human equivalent disease or disorder a network for developing a companion animal clinical trials registry could be created. The VA could move forward with supporting new collaborations to establish the relevance of dog studies to humans, both for conditions of likely future interest to the VA (e.g., posttraumatic stress disorder, natural infectious disease, Alzheimer's disease, and obesity) and for areas currently under study.

OTHER ANIMAL MODELS

The committee explored alternative animal models and non-animal alternatives in those fields where dogs have recently been (or are currently) used at the VA. Accordingly, the committee con-

sidered non-dog animal models currently used or in development for research on CVD, SCI, cancer, and diabetes. The models discussed below represent a small subset of what is available; readers seeking a more comprehensive overview are directed to recent review articles cited in the text.

Cardiovascular Disease

Although animals are used to study a wide array of CVDs, atherosclerosis and arrhythmias present especially critical domains for animal studies, with VA dog research focused on elucidating the mechanisms of heart failure and arrhythmia. Atherosclerosis (fatty deposits within the arteries) is the most common cause of heart failure but does not often present until later in life, despite the fact that atherosclerotic lesions commonly begin to form during the teenage years (PDAY Research Group, 1993). Human studies of the chronologic development of atherosclerosis typically rely on indirect means of assessment (e.g., ultrasound imaging) (Saxena et al., 2019) or a direct assessment of arterial tissue collected during autopsy; thus, no true longitudinal studies are possible in humans. In contrast, animal models have enabled the direct study of the progression of the disease (Daugherty et al., 2017). Over time, atherosclerosis can progress to ischemia and arrhythmia, with arrhythmia often serving as the fatal event for diseases of the heart (Santangeli et al., 2017; Srinivasan and Schilling, 2018). Understanding atherosclerosis and arrhythmia would go a long way toward elucidating many of the heart pathologies that affect veterans.

Rodents

For decades, the majority of experimental studies of atherosclerosis have been conducted in mice. The mouse's short life span, genetic makeup (including similarities in genes associated with increased risk in humans), ease of breeding, and low cost have made it a favored model (Getz and Reardon, 2012). The tractability of the murine genome is also advantageous; because mice are naturally resistant to atherosclerosis, genetic manipulation of their lipid metabolism is necessary (Meir and Leitersdorf, 2004). *ApoE-/-* mice, generated in 1992 (Piedrahita et al., 1992), develop total plasma cholesterol levels three- to eight-fold higher than their wild-type counterparts on a regular "chow" diet, with even higher levels on a Western diet (Nakashima et al., 1994; Plump and Breslow, 1995), and they demonstrate advanced atherosclerotic lesions by 8 to 10 months of age (Reddick et al., 1994). Another valuable model for development of atherosclerosis is the low-density lipoprotein (LDL)-receptor-deficient mouse (Ishibashi et al., 1993). Both mouse models have made significant contributions to our understanding of atherosclerosis (reviewed in Getz and Reardon, 2016a,b).

There remain some critical differences between mouse models of atherosclerosis and humans; these include the identity of the primary lipoprotein, the diversity of lipoprotein, and the failure of mice to express cholesteryl ester transfer protein (CETP), a target of interest for reducing cardiovascular risk (Davidson et al., 2009; Kosmas et al., 2016; Tanigawa et al., 2007). Mice differ from humans in the distribution of lesions, the frequency of plaque ruptures, plaque size and composition, biological processes, and mechanics (Schwartz et al., 2007). Efforts are under way to develop a murine model that mimics the plaque instability seen in humans (van der Heiden et al., 2016).

Rabbits

Before the availability of genetically modified mice, New Zealand white rabbits (NZWRs) were the primary model for experimental atherosclerosis research (Shim et al., 2016). Rabbit models helped reveal the significant role of plasma cholesterol in the etiology of atherosclerosis as

well as the importance of differently sized lipoproteins (Nordestgaard and Zilversmit, 1988). Unlike mice, rabbits transport significant fractions of cholesterol via LDL (Fan and Watanabe, 2000; Lee et al., 2017); in this regard, their lipid metabolism resembles that of humans. With regard to their expression of CETP, a possible target for reduction of cardiovascular risk, rabbits resemble humans more closely than do either mice (Lee et al., 2017; Wang et al., 2017) or dogs (Guyard-Dangremont et al., 1998; Tsutsumi et al., 2001). Other advantages of rabbit models include their relatively larger size compared with mice, which affords more tissue for analysis and enables the implantation of stents (Daugherty et al., 2017). Like mice, rabbits do not exhibit spontaneous plaque rupture. However, due to their larger size, rupture can be more readily induced (van der Heiden et al., 2016). Nonetheless, rabbits have key differences from humans, both in the location and composition of their lesions, and the small size of the animals' arteries limits the ability to use catheter-based or non-invasive imaging.

NZWRs fed a high-fat/high-cholesterol diet are the most commonly used rabbit model in atherosclerosis. In certain genetic models or with the use of mechanical manipulations, lesions will form in coronary arteries (Fan and Watanabe, 2000; Shiomi et al., 2003; van der Heiden et al., 2016). Like mice, NZWR models differ from humans in their primary circulating lipoprotein. The use of NZWRs is further complicated by their low concentration of plasma apoA-II, high levels of which are associated with susceptibility to atherosclerosis (Castellani et al., 2001), as well as the long period of high-cholesterol feeding required to induce atherosclerosis, which results in jaundice and fatty livers (Daugherty et al., 2017). Another popular model, the Watanabe hereditary hypercholesterolemic (WHHL) rabbit, lacks LDL receptors, resulting in increased plasma LDL and the eventual development of atherosclerotic markers in the coronary arteries and aorta (Buja et al., 1983). On an enriched diet, this model develops lesions similar to those observed in human familial hypercholesterolemia (FH) (Atkinson et al., 1989; Phelan et al., 1985). The recently developed *ApoE* knockout (Ji et al., 2015) and lipoprotein receptor (*Ldlr*) knockout (Lu et al., 2018) rabbit models will likely prove valuable in future studies of atherosclerosis.

Pigs

Compared to mice and rabbits, pigs share more characteristics with humans with respect to physiology and lipoprotein profile, the location and mechanics of lesion development, and the opportunity for non-invasive measurement of arteries, while providing ample tissue for analysis (Lee et al., 2017). The pig genome is more similar to humans than is the mouse genome (Wernersson et al., 2005). Nonetheless, a number of significant differences remain. For example, pig plasma, like that of mice, has very low levels of CETP activity (Guyard-Dangremont et al., 1998).

When normal pigs are fed a high-cholesterol diet, they develop hypercholesterolemia and atherosclerotic lesions similar to those in humans (Granada et al., 2009). The domestic crossbred farm pig (*Sus scrofa domestica*) fed such a diet is the most common porcine model for atherosclerosis (Granada et al., 2009). This pig develops increased plasma LDL cholesterol and exhibits simple human-like lesions at 30 weeks (Chatzizisis et al., 2008). More complex lesions resembling human coronary plaques (with calcification and hemorrhaging) can take up to 2 years to develop, during which time the pig reaches a mean body mass of more than 200 kg (Prescott et al., 1991). The unwieldiness and cost of this large pig have hindered its use for research. In 2010 a downsized version (containing a natural mutation in the LDL receptor genes) was adopted for atherosclerosis research. This animal, called the familial hypercholesterolaemia Bretoncelles Meishan pig or "minipig," when fed a high-cholesterol diet with cholic acid, rapidly develops lesions, either spontaneously or following balloon injury (Thim et al., 2010). The lesions resemble those seen in humans, including some that are vulnerable to rupture (Shim et al., 2016).

Diabetes significantly increases the risk for atherosclerotic disease, and the development of animal models to explore this risk has been a priority. In 2001 DM was superimposed (via injection of a pancreatic cytotoxin) on a domestic pig model fed a hypercholesterolemic (HC) diet. This reduced insulin-producing pancreatic β cells by more than 80 percent (Gerrity et al., 2001). The combination of DM and HC resulted in multiple severe, complex lesions with human-like morphology at 9 months (Gerrity et al., 2001; Mohler et al., 2008; Wilensky et al., 2008; Zhang et al., 2003).

Genetically engineered porcine models represent the newest tools in the domain of experimental atherosclerosis. For example, a porcine model of FH was created by inactivation of the low-density *Ldlr* gene in Yucatan miniature pigs. *Ldlr*-/- homozygotes developed severe hypercholesterolemia lesions in coronary arteries and aorta, and the disease severity was increased with a high-fat/high-cholesterol diet (Davis et al., 2014). Another Yucatan miniature pig FH model was created through the introduction of the human proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gain-of-function (D374Y) mutation (Al-Mashhadi et al., 2013). Neither of these engineered models showed evidence of plaque rupture, but given the rapid evolution of gene-editing techniques, future porcine models may surmount this shortcoming.

Given the VA's use of dogs to study arrhythmia, the committee was curious about the extent to which pigs have been used to model specific mechanisms associated with this disorder. As noted earlier in this report, while the pig's coronary anatomy resembles that of humans, certain electrophysiological differences—including the pig's transmural Purkinje system, which is not present in man or dog—can make the pig a questionable choice for research on acute ischemia. Nonetheless, pigs have been used successfully to model acute ischemia as well as heart failure, heart attack, and resuscitation (Piktel and Wilson, 2019). Pigs have also been used for modeling gene therapy strategies to treat atrial fibrillation (AF) and to reduce the susceptibility to ventricular tachycardia after heart attack (Greener et al., 2012; Liu and Donahue, 2014).

Two recently published reviews compare the various large animal models, including goats and sheep, currently used in arrhythmia translational research (Clauss et al., 2019; Piktel and Wilson, 2019). In a detailed analysis, Clauss et al. (2019) recommend pigs as the primary choice for studying myocardial ischemia and atrial tachycardia and suggest further research to characterize pigs as models for ventricular tachycardia. The authors conclude by suggesting a "practical trio" of three species for most arrhythmia research: mice for initial investigations and genetics, rabbits for electrophysiology and validation of initial findings, and pigs for translation and preclinical testing.

Spinal Cord Injury

Considerable investment in research on SCI has advanced the fundamental understanding of the cellular mechanisms guiding injury and recovery as well as the response to therapeutic interventions on both cellular and whole-animal levels. Nonetheless, for research aimed at the recovery of sensory and motor function, there has been limited translation of successful results from animals to humans (Floyd, 2019; Moore, 2019). Various laboratory animal models have been employed, and one human trial is recruiting participants as of the writing of this report (NLM, 2019).

Rodents

Both rats and mice have been vital for understanding the fundamental cellular mechanisms and systemic responses involved in recovery from investigator-induced trauma (Cheriyan et al., 2014). These studies enable the standardization of injury as well as a detailed and invasive measurement of recovery. Acute or partial SCI that impairs breathing can be investigated in rodents, but there are limits. Long-term studies are possible for lower (thoracic and lumbar) SCI, but studies of cervical SCI are limited by the difficulty (and ethics) of maintaining these animals on long-term

ventilator support (Alilain, 2019). Additionally, unlike injuries induced in the laboratory, the precise causes and manifestations of SCI in humans are of course diverse. Likewise, human genetics is more diverse than that of inbred rodents. Due to these and other constraints, the translation of SCI treatments directly from rodents to humans, in the absence of a large-animal intermediate, has been rare (Floyd, 2019; Guest, 2019; Jeffery, 2019). Nonetheless, the use of theophylline to stimulate respiration can be attributed to rodent studies (Alilain, 2019), Schwann cell transplantation has moved from rodent studies to human trials (NLM, 2019), and there is hope for other pharmacologic or genetic approaches.

Pigs

The pig spinal cord is similar to that of humans in size and anatomy, making the pig an attractive model for testing surgical and other interventions (Guest, 2019). As with rodents and all other laboratory animals, long-term maintenance that requires ventilator support is not feasible. This, in concert with the continual growth of the pig, renders pigs of little use for studying long-term recovery from respiratory compromise. Nonetheless, as described in Chapter 3, the Yucatan minipig is proving to be a useful large-animal bridge from rodent to human studies and an alternative to laboratory dogs. One example is the transplantation of Schwann cells to facilitate spinal cord regeneration, which was shown to be successful first in rodents and then in pigs (Santamaria et al., 2018), prior to the initiation of human clinical trials.

Cancer

Rodents

The mouse is one of the most commonly used small animal cancer models. As just one example of a mouse that was genetically engineered to closely resemble human disease progression, the KPC pancreatic cancer mouse model was introduced in 2005 (Hingorani et al., 2005). Compared with other mouse models, KPC mice possess more clinical and histopathological features akin to humans, making it the gold standard murine model for preclinical pancreatic cancer research (Lee et al., 2016). There are dozens of genetically engineered mouse models of melanoma; the majority contain oncogenic mutations in the pathways most likely to be altered in human disease (RAS/RAF/MEK/ERK and phosphoinositide-3 kinase), while others carry mutations in genes regulating cell cycle progression (reviewed in Perez-Guijarro et al., 2017). Mouse models have made significant contributions to the understanding of the molecular basis of melanoma formation. They are also being used in the discovery of biomarkers for both diagnosis and response and in the preclinical testing of drug efficacy as well as to investigate the mechanisms of drug resistance. Importantly, genetically altered mice currently represent the only preclinical platform for the development of melanoma immunomodulatory therapies (Day et al., 2015).

Rats are also commonly used as a preclinical cancer model; their larger size makes them better suited for studies that require imaging or surgery. Rats are frequently the model of choice for studies of colon and bone cancers (Rubio, 2017; Zhang et al., 2019). Rodent models of cancer have limitations, however. Because humans live up to 50 times longer than mice and are 3,000 times larger, with proportionately more cells, humans undergo 10^5 more cell divisions in a lifetime than mice (Rangarajan and Weinberg, 2003), potentially resulting in fundamentally different risk profiles (Tomasetti and Vogelstein, 2015). Many rat models do not mimic certain human cancer pathologies (Szpirer, 2010).

Rabbits

Rabbits are also used as cancer models; however, their use is often limited to a single type of cancer and has important limitations. For example, the rabbit VX2 model developed by Rous and Beard (1935) (Kidd and Rous, 1940) is used to study hepatocellular carcinoma (Ko et al., 2001; Parvinian et al., 2014). Tumors are induced in the liver by injecting virally infected VX2 carcinoma cells. The tumors exhibit a high rate of spontaneous necrosis, confounding studies of treatment response (Parvinian et al., 2014), although modified lines with greater viability have been developed (Pascale et al., 2012). Rabbit models have also been used in studies of metastatic colorectal cancer (Prieto et al., 2017), oral cancers (Chen and Lin, 2010), skin cancers (Breitburd et al., 1997), and breast cancer (Zhang et al., 2017).

Pigs

In general, cancer cell biology in pigs and dogs is more analogous to that in humans than are small animal models, while the larger animals' size and anatomy make them well-suited for interventional studies, including device testing and surgical practice (Flisikowska et al., 2013; Gardner et al., 2016).

Pigs offer a large-animal alternative to laboratory dog models, with outbred populations displaying genetic diversity akin to human populations (Schachtschneider et al., 2017a), thereby sidestepping the inbreeding critique leveled at domestic dogs. Pigs' many similarities to humans (in terms of cancer pathophysiology as well as size, anatomy, genetics, and epigenetics) as well as their low cost compared to primates (Groenen et al., 2012; Schook et al., 2005; Swindle et al., 2012) make them attractive subjects. Pigs age at three to five times the rate of humans but can live up to 10 years; this enables the relatively rapid detection of disease while allowing researchers to monitor tumor progression over long time periods (Flisikowska et al., 2013; Watson et al., 2016). Anatomical similarities with humans facilitate the use of imaging technologies (positron emission tomography, computed tomography, magnetic resonance imaging) developed for humans (Sieren et al., 2014). Pig anatomy allows drugs to be administered via the same routes used in humans, and pig blood can be drawn in sufficient size and frequency to enable pharmacokinetic analysis. Pig cytochrome P450 enzymes metabolize four of the six most common probe substrates with activities similar to those of human cytochrome P450, thereby supporting the use of pigs for a range of drug metabolism studies as well (Schelstraete et al., 2019). Comorbidities common in human disease (e.g., nonalcoholic steatohepatitis, alcoholic cirrhosis) can also be induced in pigs (Gaba et al., 2018; Lee et al., 2009).

Genetically engineered pig cancer models exhibit disease courses similar to those observed in humans. The Oncopig cancer model (OCM) is a notable recent example (Schook et al., 2015). The OCM can be made to develop site-specific tumors via the induced expression of *KRAS*^{G12D} and *TP53*^{R167H} transgenes, an oncogene and a tumor suppressor, respectively, both of which are commonly observed in human cancers (Schook et al., 2015). Schachtschneider and colleagues (2017a) outlined a number of unmet clinical needs that OCM research could potentially fill. For example, in the quest to improve early detection of cancers, the OCM offers an ideal platform to investigate the prognostic value of candidate cancer biomarkers in liquid biopsies. Thanks to similarities between OCM and human metabolic pathways, the OCM is also a strong candidate for exploring drug dynamics, kinetics, and toxicity.

The OCM has been used to model a variety of both hematologic and solid tumor cancers, including soft tissue sarcoma, or STS (Diaz et al., 2016; Schachtschneider et al., 2017a,b; Schook et al., 2015). An OCM STS model was used to test the efficacy of a real-time, image-guided technique to precisely place catheters in tumors for thermal ablation (Schachtschneider et al., 2017a). A

porcine osteosarcoma model is being studied to obtain mechanistic insights into human bone cancer (Saalfrank et al., 2016), and models for pancreatic and other cancers, currently under development, may provide tools that lead to earlier diagnosis as well as new surgical interventions (Bailey and Carlson, 2019; Diaz et al., 2016; Kalla et al., 2020; Leuchs et al., 2012; Schachtschneider et al., 2017a). Pig size and anatomy also make the pig an attractive model for liver cancer. OCM hepatocarcinoma cell lines recapitulate human hepatocellular carcinoma (HCC) and may prove useful for developing immunotherapy. The antitumor immune response in the OCM includes both innate and adaptive recognition of induced tumors as well as tumor-induced suppression of T-cell effector functions, all of which are relevant to the human condition (Overgaard et al., 2018).

Given the VA's interest in melanoma, the committee reviewed pig models for this most deadly form of skin cancer. Hereditary metastatic melanoma is modeled by three strains of miniature pig, most recently the melanoma-bearing Libechof minipig (MeLiM) (Bourneuf, 2017). While the MeLiM tumors display histological, immunohistochemical, and hematological similarity to human melanomas, they also show significant differences, including their non-UV-dependent origin and a higher rate of spontaneous regression (although a higher rate of melanoma progression is observed in MeLim than in the two other strains) (Horak et al., 2019). The availability of the OCM, with its potential to generate a variety of cancer types (including melanoma) through targeted mutation, opens up new possibilities for using pigs to model melanoma at all stages of the disease, from tumorigenesis to pathogenesis to therapeutics (Cuoto et al., 2019).

Non-Mammalian Models

In addition to the mammalian models mentioned above, it should be noted that non-mammalian species, including fruit flies and a variety of fish, play significant roles in the elucidation of genetic pathways associated with tumor development (Cagan et al., 2019). Recombinant zebrafish (*Danio rerio*) are used to study the genetic basis of melanoma initiation and progression and are also used in the validation of targeted treatments (Cuoto et al., 2019; Fernandez Del Ama et al., 2016). Nonetheless, zebrafish have limited utility for phenotypic modeling of human cancers (Schachtschneider et al., 2017a).

Diabetes

While laboratory dogs are no longer used in large numbers to study diabetes (and published research is more likely to use companion dogs with naturally acquired disease), they continue to be employed in targeted studies that rely on their physiology and similarity to particular human disease states.

Rodents

Rodents have been the primary species for experimental diabetes research for many years (King and Bowe, 2016; Rees and Alcolado, 2005). The non-obese diabetic (NOD) mouse and the biobreeding diabetes-prone rat have been favored (Yang and Santamaria, 2006) for type 1 diabetes, while the $Lep^{ob/ob}$ mouse (deficient in leptin), and the $Lep^{db/db}$ mouse and Zucker Diabetic Fatty rat (both deficient in leptin receptor) are commonly used in the study of type 2 diabetes (King, 2012). Many factors that make rodents attractive models for cardiovascular disease research, such as their low cost and rapid reproduction, also apply in the context of diabetes. Mouse models have provided valuable insights into diabetic disease mechanisms and treatment by, for example, identifying signaling pathways and genetic factors that can lead to type 1 diabetes (Driver et al., 2012; Wallis et al., 2009) and testing type 2 diabetes therapies (Gault et al., 2011; Park et al., 2011).

Pigs

Pigs share many anatomic, metabolic, and pathophysiologic traits with humans that render them useful for studying the complex factors involved in the initiation and progression of diabetes. Adult domestic pigs are used on occasion (e.g., for testing medical devices scaled for adult humans and when large blood volumes are needed). However, minipigs are generally preferred for their smaller size. The domestic pig's beta-cell-mass-to-body-mass ratio—considered a good parameter for interspecific comparisons—shows a trajectory over the lifetime of the animal that closely resembles the ratio in rats (Bock et al., 2003; Montanya et al., 2000), and minipigs have been used to study the processes leading to beta-cell dysfunction (Larsen, 2009; Larsen et al., 2005). Obesity is reliably induced in pigs through diet, and genetic engineering has produced strains of pigs tailored to particular applications in diabetes and dyslipidemia research (Kleinert et al., 2018).

A variety of approaches have been used to create tailored pig strains (Cho et al., 2018; Kleinert et al., 2018; Phelps et al., 2003; Renner et al., 2010; Wang et al., 2015; Yum et al., 2016). Inbreeding can produce genetically standardized models; outbreeding can produce populations of pigs with genetic variation akin to that observed in human populations. Type 2 diabetes has been modeled in pigs by the introduction of a glucose-dependent insulinotropic polypeptide (GIP) receptor mutation that suppresses insulin secretion in response to food uptake and results in reduced glucose tolerance, reduced insulin secretion, and decreased pancreatic beta-cell mass (Renner et al., 2010). This model was used to test a promising drug, liraglutide, which compensates for the GIP deficiency by increasing downstream signaling (Renner et al., 2016; Streckel et al., 2015). Drug studies in pigs produced more consistent results than studies performed in rodents, and the pharmacokinetics in pigs more closely resembled that of humans (Renner et al., 2016; Tamura et al., 2015).

Islet transplantation is considered a potentially useful therapy for type 1 diabetes; however, shortages of human donor organs preclude widespread adoption of the protocol. Xenotransplanation has been put forth as a possible solution and is a subject of active research with recognized risks (Denner et al., 2016; FDA, 2016; Spizzo et al., 2016). There is currently much interest in developing genetically engineered pigs from which islets could be harvested, which would be done shortly after birth to reduce the risk of zoonotic infection (Cooper and Ayares, 2011). Neonatal pig islets offer additional advantages, including reduced immunogenicity, increased in vitro stability, and the tendency to proliferate better after isolation (Vanderschelden et al., 2019).

Summary

While animal studies have made notable contributions to understanding CVD pathways and uncovering therapeutic targets (Daugherty et al., 2017; Getz and Reardon, 2012; Nishida et al., 2010), all models have limitations, making the choice of animal dependent on the precise question being asked. For example, while plaque calcification (a significant component of coronary artery disease) is common in humans, it is minimal in mice (Otsuka et al., 2014). Dogs are generally resistant to atherosclerosis, although it can be induced in the laboratory with a special diet (Moghadasian et al., 2001). In contrast, dogs arguably display the strongest physiological similarity to humans for a particular, relatively small suite of cardiovascular research needs related to arrhythmia (Nishida et al., 2010).

Despite the variety of approaches currently available for studying SCI, there is no laboratory animal model that mimics the diversity of injuries and the diversity of the human population. Long-term recovery from SCI that compromises respiration cannot be studied in animals due to the practical and ethical concerns regarding the maintenance of an immobilized, sedated, or paralyzed laboratory animal for long periods. This type of SCI is nonetheless common in humans, and its impact is devastating. Indeed, the suffering that human survivors of cervical SCI must endure is too

distressing to be considered humane for laboratory animals. Advancing these studies will require an accelerated effort to conduct human trials, even pilot studies, for interventions that cannot be tested on large animals in the laboratory.

Laboratory dogs have largely been supplanted by other animals, primarily mice, for the study of cancer. The same holds true for diabetes, although dogs remain the most well established model for the quantification of liver glucose uptake, which cannot be measured directly in humans or rodents (Kleinert et al., 2018). Recent developments enabling targeted gene editing in pigs show promise for improved translatability to the human disease state.

Conclusion 4-3: With respect to other animal models, rats and mice are the predominant species used for biomedical research in the fields of cardiovascular disease, spinal cord injury, cancer, and diabetes. For studies that cannot be performed in rodents (due to constraints of size, anatomy, or physiology), the pig has become the large animal translational model of choice. While pigs are not tractable for all areas, their potential uses are likely to expand in the near future as genetically modified strains become more widely available.

NON-ANIMAL MODELS: NEW APPROACH METHODOLOGIES

The transition from the exclusive use of animal models to an increasing reliance on NAMs requires first establishing confidence that NAMs can adequately address the scientific questions being posed. In this context, while NAMs are not yet able to recapitulate the complexity of whole animal systems, some are now capable of interrogating specific aspects of biology that are confined to a limited physiological space (at the molecular, cellular, or organ level) and that take place in well-defined contexts of use.

Advances in the cultivation and differentiation of human embryonic and induced pluripotent stem cells are providing ready access to one of the most important tools known to biomedical research, enabling tissue-, disease-, and even patient-specific modeling using a range of in vitro systems. Concurrently, the fields of three-dimensional (bio) printing and biomedical engineering are providing advanced scaffolds (i.e., micro physiological systems) to support cellular systems with physiological complexity never before observed outside the human body. The vast amount of biological information being generated by these and other innovative technologies has in turn fueled the development of new computational tools using the power of machine learning/artificial intelligence and in silico modeling. In addition to in vitro and computational approaches, significant advances are also being made in the ability to preserve and use organs and tissues from human donors. Although none of these technologies can currently "replace" the dog, they do offer the potential to provide more human-relevant mechanistic insights and may therefore warrant consideration as valuable resources for VA research.

Here the committee provides a few examples of existing or emerging technologies that could potentially be used to address biomedical research needs identified by the VA in the areas of CVD, SCI, and cancer. These examples are not intended to be comprehensive, but rather to highlight some of the remarkable advances being made in fields as complex as in vitro biology, computational modeling, and ex vivo use of human organs, with relevance to VA clinical research interests. Several of the NAMs currently under development may be capable of addressing certain clinical research needs of the VA, but their evaluation and adoption will require a coordinated effort from both NAM developers and VA researchers.

Cardiovascular Disease

There is a growing arsenal of non-animal tools and approaches with potential applicability to the study of cardiovascular function, disease, and treatment. A subset of these tools are described here, with a focus on areas of VA research relevant to this report: AF, premature ventricular contractions, cardiac contractility, and cardiomyopathy.

Stem Cells

Human pluripotent stem cell–derived cardiomyocytes have revolutionized the field of cardiovascular research. Methods for differentiating human induced pluripotent stem cells (hiPSCs) into beating cardiomyocytes (hiPSC-CMs) have been standardized and commercialized, providing the research community with wide access to these tools. However, most hiPSC-CMs used in research remain predominantly in an immature state with regards to their physiological structure and function, demonstrating fetal gene expression as well as morphological, metabolic, and contractile characteristics that differ from those of adult cardiomyocytes (Machiraju and Greenway, 2019). As a result, disease modeling using hiPSC-CMs may be of limited value if the cells are not adequately characterized with respect to their electrophysiology, contractility, kinetics, etc. Fortunately, rapid progress toward more physiologically relevant systems continues to be made, with, for example, recent success in the maturation of hiPSC-CMs using a diverse array of approaches (Machiraju and Greenway, 2019).

Researchers have used human embryonic stem cells (hESCs) to generate confluent sheets of atrial-like cardiomyocytes (hESCs-CMs) with spontaneous pacemakers, allowing for the induction of abnormal rhythms (i.e., simulated AF) using chemicals or hormones with pacemaking restored to normal rhythm after the stimulus is removed (Laksman, 2019; Laksman et al., 2017). Voltage-sensitive dyes make it possible to map out the electrical signals as they propagate through the tissue in a spiral pattern. Using this system, researchers have demonstrated that antiarrhythmic drugs modulate the rotor activation patterns in a manner consistent with their known efficacy in treating and preventing AF.

Organoids and Microphysiological Systems

The ability to direct the differentiation of hiPSCs into specific tissues has been foundational to the development of organoids, which are three-dimensional (3D) structures of self-organizing, tissue-specific cells that can recapitulate the physiological functions of human tissues far better than two-dimensional (2D) cultures of the same cells (Mills et al., 2017; Park et al., 2019b). The construction of organoids that use iPSC-CMs from human patients with known genotypes and phenotypes of clinical interest has enabled the development of more physiologically relevant in vitro tools for modeling a spectrum of conditions, from disease pathophysiology to drug screening (Mills et al., 2017; Nugraha et al., 2018). A further extension of this approach has been the development of engineered 3D microphysiological systems (tissue or organ chips) that support the integration of multiple cell types into an organ-like configuration, with the goal of obtaining more organ-like functions. Researchers have developed numerous 3D working models of human heart tissue (Feric et al., 2019; Guyette et al., 2015; Kitsara et al., 2019; Noor et al., 2019), including an intact scale model of a human ventricle (MacQueen et al., 2018), which offer the potential to study heart disease as well as drug safety and efficacy in patient-derived cells.

Computational Models

Physiologic computer models of the human heart are proving to be an effective and efficient alternative to animal-based experimentation in understanding and predicting cardiac adverse events during drug development (Passini et al., 2019) and have been used to develop virtual in silico drug trials incorporating variability in response seen across an entire population of patients (Britton et al., 2013; Passini et al., 2017). Physiologic computer models are also being developed to enable patient-specific diagnosis and treatment of cardiovascular disease. As noted in a recent review (Gray and Pathmanathan, 2018, p. 82):

> Patient-specific computational fluid dynamic models are being used to address aortic aneurysms (Vorp, 2007), coronary stenosis (Taylor et al., 2013), cardiac valves (Votta et al., 2013), and congenital heart disease (Pittaccio et al., 2005; Corsini et al., 2014). Bi-ventricular patient-specific models of electromechanics have been applied to heart failure (Aguado-Sierra et al., 2011; Krishnamurthy et al., 2013; Kayvanpour et al., 2015), left ventricular assist devices (Smith et al., 2011), and cardiac resynchronization therapy (Niederer et al., 2011; Sermesant et al., 2012; Crozier et al., 2016). Patient-specific models of electrophysiology have shown promise in regard to genetic mutations (Hoefen et al., 2012), ablation therapy (Smith et al., 2011), and clinical classification criteria (Galeotti et al., 2013).

Stem Cells and In Silico Modeling for Regulatory Use

The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative was established to update the paradigm for assessing the proarrhythmic risk of pharmaceuticals, with broad applicability to other sectors (Blinova et al., 2018; Colatsky et al., 2016; Park et al., 2019a). This project seeks to develop a standardized in silico version of a human ventricular cell in which to evaluate the risk of cardiac toxicity. The evaluation of proarrhythmic risk is based on an electrophysiologic understanding of proarrhythmia with two primary components: (1) in vitro drug effects on multiple cardiac channels plus in silico reconstruction of cardiac action potential, and (2) confirmation using human stem cell–derived cardiomyocytes.

The U.S. Food and Drug Administration's (FDA's) Division of Applied Regulatory Science has a goal of moving new methods, including alternatives to animals, into the drug review process (FDA, 2019). This includes the development of in vitro cellular microsystems for the early stages of drug testing, with cardiac contractility as one example of a functional endpoint that can predict drug effects (Ribeiro, 2019; Ribeiro et al., 2015). These systems use micropatterning of hiPSC-CMs on engineered material that enables the contractility of the muscle fibers to be used as a physiologically relevant endpoint of cellular function downstream of an action potential.

Human Heart Tissue

Although laboratory animals provide a convenient and consistent medium for research into mammalian biology, the historical difficulty of translating these findings into (human) clinical practice is well documented and universally acknowledged. When considering approaches to improve translational impact and efficiency, the use of intact human tissues (in situ or ex vivo) offers perhaps some of the best opportunities. The use of human donor hearts not suitable for transplantation and those from patients with end-stage heart failure receiving donor hearts offers an extremely valuable translational platform for advancing research on human heart function and disease (Efimov, 2019; Gloschat et al., 2016). The use of human hearts for biomedical research,

as with all human tissues, will become even more impactful as processes for their procurement, distribution, and storage continue to improve.

The physiology and cellular integrity of human hearts maintained ex vivo deteriorates rapidly once the heart is removed from the host. However, thin slices of freshly harvested heart tissue offer a promising model of intermediate structural/mechanical complexity with the innate 3D tissue architecture and extracellular matrix preserved (Kang et al., 2016). This allows intact myocardium to be used for a wide range of research purposes, from the visualization of 3D collagen distribution and micro/macrovascular networks to probing the effects of novel conductive biomaterials on cardiac physiology and testing pharmacological safety and efficacy (Watson et al., 2019a,b). Thin slices preserve normal electrophysiology, enabling the testing of pharmacological interventions, gene therapy, cellular therapies, and medical devices, among others (Kang et al., 2016). Although they are much shorter-lived than slices, larger (wedge) preparations of intact heart tissue have also proved useful. Wedges of human ventricles were used to optimize thermal ablation techniques for creating lesions to block reentrant wavefronts, thus mitigating the tachycardia that can lead to ventricular fibrillation and sudden cardiac death (Sulkin et al., 2018).

Using image-based analysis and multicellular mathematical modeling of electrical activation, Stephenson et al. (2018) showed the 3D disposition of the cardiac conduction system for the first time in an intact human heart with congenital atrioventricular septal defects. The advance raises new possibilities for understanding arrhythmogenesis and ablation strategies by studying the congenitally malformed heart. A high-throughput left ventricular myocardial slice model has been developed that is physiologically stable for at least 3 days, enabling the investigation of ion signaling and myofiber contraction at scale (Thomas et al., 2016). More recently, a medium-throughput methodology retained physiological functionality in both pig and human heart slices for up to 6 days (Ou et al., 2019). The heart slice model has been further refined using electromechanical stimulation to prevent the onset of myocardial dedifferentiation that begins to occur when the tissue slices are placed in culture (Watson et al., 2019a), bringing researchers another important step closer to recapitulating the complexity of the in situ human heart.

Spinal Cord Injury

Bioprinting

The complexity of the central nervous system presents a significant challenge to the construction of non-animal systems for studying SCI. However, advances in 3D bioprinting are beginning to overcome these constraints, enabling the study of neuroregeneration in a context that is faithful to the anatomy and biochemistry of the human condition (Joung et al., 2020). Bioengineered scaffolds are being developed for implantation into the site of injury and for stimulation of central nervous system regeneration (Koffler et al., 2019); they also offer broad research applications (Joung et al., 2020). Spinal cord spheroids, bioprinted into 3D hydrogel, can be mass produced for use in the preclinical safety and efficacy screening of pharmacological interventions (Bowser and Moore, 2020).

Microphysiological Systems

Even more complex than regeneration, the locomotion circuit requires multiple cell types organized in a precise arrangement and capable of acting in a coordinated fashion. No non-animal model has yet succeeded in replicating this motor circuit. However, the combination of compartmentalized

microfluidic culture with 3D culture techniques and the use of hiPSCs may ultimately produce a model capable of mimicking human neuromuscular disease in vitro (Badiola-Mateos et al., 2018).

Organotypic Modeling with Ex Vivo Tissue

Recent studies have obtained physiologically relevant data using ex vivo spinal cords from rodents. Adult mouse spinal cords were used to identify populations of axons recruited by spinal cord stimulation, a method employed to reduce chronic pain in the clinic (Idlett et al., 2019). In a study by Pandamooz et al. (2019), slices of adult rat spinal cord were maintained in culture for 1 week prior to being subjected to mechanical damage simulating an SCI. Treatment of damaged slices with valproic acid, a drug that shows potential for promoting SCI recovery in the clinic, reduced expression of the inflammatory cytokine TNF-a and increased expression of the neurotrophic factor BDNF (Pandamooz et al., 2019), supporting the potential utility of slice models for studies of pathophysiology and drug screening.

Computational Modeling

Computational models are being developed to study discrete aspects of SCI, such as pressure ulcer formation (Ziraldo et al., 2015) and cough stimulation (Pitts et al., 2016). It should be noted that the development of these models involved validation against biological data, which was collected in humans with SCI for the first study and in cats for the second.

Cancer

Microphysiological Systems and Organ Chips

Organ chips have been used to model multiple steps in the cancer cascade, from tumor growth and angiogenesis to the epithelial–mesenchymal transition, invasion, and metastasis. Organ chips enable the growth of multiple tissue types on layers of extracellular matrix seeded on a flexible, see-through substrate under conditions of constant perfusion, with a medium or blood flowing through an endothelium-lined vasculature (Sontheimer-Phelps et al., 2019). Partitions within the device enable researchers to study cell migration and cell–cell communication, including trans-endothelial migration of immune cells. Organ chips have enabled the recapitulation of complex drug-resistance profiles seen in vivo, which do not find expression in more simplified in vitro systems (Sontheimer-Phelps et al., 2019).

Organ chips and related biomimetic systems have begun to make significant contributions to the study of melanoma. In a 2D model, metastatic melanomas demonstrated directed migration when co-cultured with epithelium or fibroblasts from different organs, providing an avenue for interrogating the factors responsible for organ tropism (Zhang et al., 2015). Melanoma cell growth and morphology are strongly dependent on the mechanical properties of the substrate (Prauzner-Bechcicki et al., 2015). One 3D melanoma model recapitulates a stratified epidermis, human extracellular matrix, and blood and lymphatic capillaries; this model has shown promise for testing new anticancer compounds in vitro (Bourland et al., 2018). In time it should be possible to model metastatic spread by combining multiple fluidically linked organ chips to create human "body-on-chip" models (McAleer et al., 2019; Sontheimer-Phelps et al., 2019).

In comparing organ chips to animal models, it is worth noting that as cancer treatment becomes increasingly specific—for example, with the use of therapeutic monoclonal antibodies—the ability to evaluate therapies in non-human species becomes more challenging (Sontheimer-Phelps et al.,

2019). Multi-organ models built on organ chips offer a possible way forward. NIH is contributing to this effort on several fronts, including NCI's Cancer Tissue Engineering Collaborative Research Program as well as a grant instrument supporting research to test the utility of incorporating "tissue chip" models into clinical trial design (HHS, 2019; NCI, 2019).

Organ chip technology is still young, relatively expensive, and not as well characterized as other 3D culture systems. The successful incorporation of organ chip systems into research will therefore require dedicated and innovative efforts to establish confidence in these systems. As new commercial sources become available, however, their technical robustness is expected to increase and their costs to decrease (Sontheimer-Phelps et al., 2019).

Computational Modeling

In the realm of cancer, a major aim of computational modeling is to improve the success of drug trials by streamlining the process of translation (McKenna et al., 2018). Researchers are pursuing this goal through multiple avenues. For example, a 2018 study developed simulations of nanoparticle flow through blood vessels in order to help drug designers predict the optimal particle shape for drug delivery; real-world experiments validated these predictions (Shah et al., 2018).

Patient-to-patient variability in drug response presents a critical challenge for cancer treatment. In one case a model of breast cancer, personalized with details from histopathology, imaging, and molecular profiling, showed success in simulating the responses of individual tumors to a 12-week treatment regimen (Lai et al., 2019). In another, high-resolution metabolic models of colorectal cancer cells, constructed using an RNA sequence dataset, were able to detect patterns of metabolic rewiring in the individual cancers and thereby identify three potential new drugs for colorectal cancer (Pacheco et al., 2019). The optimal approach to drug discovery for cancer will likely include a combination of computational modeling and biological testing (Nagaraj et al., 2018).

Incorporation of New Approach Methodologies at the VA

The Animal Welfare Act and associated U.S. Public Health Service (PHS) policies set forth federal requirements designed to ensure that investigators in the United States have appropriately considered alternatives to procedures that can cause more than slight or momentary pain or distress in animals.[2] Additional guidance for VA researchers and institutional animal care and use committees (IACUCs) on the evaluation of non-animal alternatives is provided in the Veterans Health Administration handbook 1200.07, *Use of Animals in Research*, which requires researchers to perform one or more database searches for alternatives and indicate if any of the animal procedures can be replaced by computer models or in vitro techniques (VHA, 2011; Appendix D section 1.v.). Although the intent of this requirement is laudable, the fact that NAMs cannot (and are not intended to) replace the physiological complexity of intact animals leaves ample opportunity to exclude their consideration if so desired.

Indeed, a review of the animal component of research protocol (ACORP) forms associated with the 14 protocols for VA biomedical research in laboratory dogs that were active as of June 1, 2017, revealed that most database searches appeared to have been conducted using narrow definitions, as opposed to casting a broad net capable of revealing all possible alternative approaches to addressing the question. A protocol review process that requires the principal investigators and IACUCs to explore broader contexts for using NAMs would serve to encourage the advancement and uptake of new promising technologies.

[2] U.S. Code 7, Chapter 54: Transportation, sale, and handling of certain animals; CFR Title 9, Subchapter A: Animal welfare. Both available at https://www.nal.usda.gov/awic/animal-welfare-act (accessed December 30, 2019).

The effective incorporation of NAMs into any given area of biomedical research will require VA investigators accustomed to animal-based research to consider reframing the scientific question, as opposed to evaluating NAMs purely based on their ability to fully replace a laboratory animal. Some questions that are currently studied in dogs and other large animals may be partially addressable with less complex systems, such as NAMs that could offer more detailed interrogation of mechanistic information germane to the research question, and even when large animal use cannot be eliminated entirely, the use of NAMS could reduce the numbers of animals required for translation. However, the appropriate evaluation and use of NAMs will necessitate collaborations among diverse research communities (in vivo, in vitro, in silico), which are not always accustomed to such interactions. Overcoming cultural and historical barriers to changing the way research is done could pose a barrier to NAM adoption if it is not sufficiently incentivized by the VA (ICCVAM, 2017).

Conclusion 4-4: While the scientific and institutional animal care and use committee review processes at the U.S. Department of Veterans Affairs adhere to all relevant policies established by the U.S. government (as described in Chapters 2 and 5), compliance with these standards on its own may not be sufficient to ensure adequate identification and consideration of new approach methodologies (NAMs). Even in the case of protocols that still require the use of laboratory animals, researchers need to be encouraged to evaluate and incorporate NAMs where feasible.

Summary of New Approach Methodologies

U.S. federal agencies are placing an increased emphasis on ensuring that researchers adequately consider alternatives to animal use (GAO, 2019). Although none of the NAMs highlighted above can serve as an immediate or complete replacement for animals, they do enable researchers to interrogate aspects of human biology that cannot be addressed in dogs or other animals and thereby offer the potential to enhance the translational impact of VA research. In cases where the current research is focused on discrete mechanisms, NAMs can address the ethical imperative to reduce the number of animals used and may eventually replace the current dog model for conducting mechanistic studies.

However, the incorporation of NAMs into any animal-based research program will not be successful without a dedicated and comprehensive effort to do so. To facilitate their adoption, institutions need to identify and address institutional and cultural practices that may impede the evaluation and adoption of NAMs. In this context, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) published *A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States* (2017), which describes the processes necessary for developing, establishing confidence in, and effectively incorporating the use of NAMs to replace or complement existing animal-based test methods. ICCVAM member agencies such as FDA and the U.S. Environmental Protection Agency have subsequently incorporated these principles into strategic plans tailored to address the particular needs of each agency and its stakeholders. The VA now has an opportunity to establish itself as a leader in expediting the incorporation of human-relevant technologies that could enhance the translational relevance of VA-funded research (shortening the time from bench to bedside) while reducing the reliance on laboratory animals and, most importantly, could improve and protect the health of veterans.

HUMAN CLINICAL TRIALS

During the time frame that this National Academies of Sciences, Engineering, and Medicine committee was charged with reviewing VA biomedical research in laboratory dogs, one protocol that had originally proposed to use laboratory dogs, which was designed to study the ability of targeted nanoporphyrin to potentiate immunotherapy for bladder cancer, was changed to a human clinical trial. The committee did not request details from the VA regarding the precise reasoning or justification for the decision to move from laboratory dogs to human trials in this instance. The committee notes the importance of always considering human clinical trials as an alternative to laboratory dogs and providing explicit justification for why human clinical trials are inappropriate to ethically meet the study objectives.

The VA ACORP forms reviewed by the committee include a number of opportunities and areas for an explanation of animal model selection but do not require a justification for excluding human clinical trials from consideration. Perhaps this justification for excluding humans appears on other research review and approval forms the committee did not see. In any case, the committee believes that human clinical trials need to be fully considered and that reasons for excluding humans need to be explained in supporting research documentation.

Recommendation 4: Develop a strategic roadmap to create, track, and sustain internal efforts to incorporate new approach methodologies (NAMs) in U.S. Department of Veterans Affairs (VA) biomedical research.

The VA should establish a strategic roadmap and accompanying framework to promote the development and incorporation of NAMs to replace, reduce, or refine the use of dogs and all other laboratory animals in VA research. This framework should prioritize:

- **Modifying the protocol review processes (see Recommendation 3) to require and support robust consideration of NAMs, human clinical trials, companion dogs, and alternative animal models. The potential of these alternatives to contribute to the overall goals of the research, not just to replace laboratory dogs, should be considered.**
- **Incentivizing the use of NAMs. Examples of ways to do this include:**
  - **Developing and funding new VA grant opportunities to promote the development of NAMs that meet the unique needs of VA researchers, including the use of human tissues and organs, in vitro, in silico, and computational approaches.**
  - **Funding for researchers and institutional animal care and use committees to undertake training in state-of-the-art, human-based methods to increase awareness and help establish confidence in these new approaches. Hands-on training and similar knowledge transfer opportunities will be particularly important and should be prioritized.**
  - **Implementing compulsory funding to promote the evaluation and optimization of NAMs that address research objectives identified in studies that currently require the use of laboratory dogs (i.e., parallel funding requirements).**

Recommendation 5: Establish long-term external collaborations to optimize the use of companion dogs and humans in biomedical research.

The U.S. Department of Veterans Affairs should prioritize the development and continuation of external multi-disciplinary collaborations to develop, validate, and apply alternatives to the laboratory dog in biomedical research. This effort should result in the following:

- **Increased collaborations with external scientists and use of public–private partnerships to promote cross-sector communication and cooperation.**
- **The fostering of collaborations with researchers conducting clinical trials in companion dogs to identify or develop trials to benefit veterans and dogs.**
- **The encouragement of the use of human organs and tissues from human organ banks whenever possible.**

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5

Care and Welfare of Laboratory Dogs Used in Biomedical Research Funded by or Conducted at the U.S. Department of Veterans Affairs

This chapter begins with a discussion of the literature surrounding the latest approaches in animal care and welfare as well as the relevant regulations and standards that apply to laboratory dog care and welfare. The chapter ends with a discussion of recommended actions for the U.S. Department of Veterans Affairs (VA) to enhance the well-being of laboratory dogs used in biomedical research.

CURRENT KNOWLEDGE IN THE SCIENCE OF ANIMAL WELFARE: A BRIEF OVERVIEW

Over the past 50 years an impressive array of work has been done to understand the welfare of animals in a range of settings, from farms and laboratories to zoos, shelters, and the wild. To survey that work, even within the laboratory setting, is beyond the scope of this report, but four key conceptual advances from this period inform practice regarding the welfare of animals in human care today. These advances reflect four developments in the field of animal welfare: the recognition of animal sentience, the emergence of three distinct approaches to the nature and assessment of animal welfare, a focus on positive and negative welfare states, and the recognition that welfare assessments incorporate both resource-based and animal-based considerations.

Recognition of Animal Sentience

The first of these developments was the widespread acceptance that many kinds of animals—and certainly all of the higher vertebrates (mammals and birds)—are sentient organisms (Duncan, 2006; Proctor, 2012; Proctor et al., 2013). They have the ability to receive sensory input that originates inside or outside of their bodies, and the integration of that input can produce states that may range from negative to positive, which the animal can consciously experience (Proctor et al., 2013; Silverman, 2008). Acceptance of animal sentience paved the way for advances in understanding the nature of animal welfare and how to assess it.

Three Approaches to the Nature and Assessment of Animal Welfare

The second development stemmed from the emergence of three distinct schools of thought on the nature and assessment of animal welfare; these schools of thought can be referred to as "biological functioning," "natural living," and "affective states" (Duncan, 2006; Duncan and Petherick, 1991; Fraser, 1995, 2003, 2008; Green and Mellor, 2011; Hemsworth et al., 2015; Mellor, 2012, 2015a,b,c). The biological functioning approach associates good welfare with the satisfaction of an animal's physical and behavioral needs and therefore focuses on ensuring survival and reproduction, the absence of disease or injury, and the absence of abnormal or pathological behaviors. The natural living approach defines optimal welfare conditions as those that approximate the animals' natural habitat as closely as possible and that enable the animals to exhibit "natural" behaviors to the fullest extent possible. The affective states approach argues that how an animal feels is of primary importance in determining its welfare, and it associates good welfare with conditions in which an animal's experience of positive emotions, feelings, and sensations outweighs the negative. Because emotions or feelings may not be directly measurable, this last approach relies on behavioral and physiological cues to indicate positive or negative affective states.

Carried to its most extreme interpretation, each of these approaches to animal welfare may result in a situation where the animal's welfare is arguably poor (Fraser, 2008). For example, a focus on biological functioning that leads to a use of extremely hygienic and restricted housing to prevent any risk of injury and illness may create such a barren or stimulus-poor environment that the animal suffers from boredom, stress, or anxiety due to separation from conspecifics. A natural living focus that allows animals to roam in large outdoor accommodations in natural social groups could expose the animals to diseases, parasites, predators, and aggression from conspecifics. And although the constant availability of unlimited rich food may cause an animal to "feel good" most of the time, satisfying the goals of the affective states approach, this can be lethal in the long run. It is also the case, however, that many types of animal welfare interventions can satisfy the goals of multiple approaches to animal welfare. For example, routine vaccination programs for canines not only provide protection from disease, but they also support better overall health, a greater propensity to behave naturally, and an avoidance of the negative affective states associated with disease, such as nausea or dehydration. Providing compatible dogs with conspecific socialization allows them to exhibit species-typical behaviors, obtain beneficial exercise, and have opportunities for pleasurable experiences such as play.

There is no scientific consensus regarding which model—biological functioning, natural living, or affective states—best represents animal welfare (Fraser, 2008). Instead, conceptual frameworks that tie these approaches together have emerged in the agricultural and scientific arenas. The five freedoms framework, proposed in 1979 by the Farm Animal Welfare Council in the United Kingdom, argues that animals in human care should be free from hunger, thirst, and malnutrition; free from discomfort; free from pain, injury, and disease; free from fear and distress; and free to express normal behavior (Webster, 2005a, pp. 12–16). Responding to some of the limitations of the Five Freedoms framework (e.g., it is impossible to ensure that animals are never hungry), Mellor and Reid (1994) developed the five domains of welfare compromise model, with a scoring system to assess welfare in each of these domains. The original five domains framework focused on measurement of welfare compromise (i.e., negative welfare states).

Positive and Negative Welfare States

The third major development in animal welfare science was a shift in focus from minimizing or eliminating animal suffering to creating conditions that enhance positive welfare states and pleasurable experiences for animals (Boissy et al., 2007; Gonyou, 1993; Mellor, 2015a,b,c; Yeates

and Main, 2008). The basic premise behind this shift was that welfare exists on a continuum of poor to good, so merely eliminating negative welfare conditions would not necessarily move an animal into a positive welfare state. In this vein, the five domains model and other approaches to animal welfare management have since been extended to consider positive welfare states (Green and Mellor, 2011; Mellor and Beausoleil, 2015). In discussing welfare assessment methods for laboratory animals, Beaver and Bayne (2014) suggest that indicators of poor welfare be addressed first, before considering indicators of positive welfare. Certainly, a serious indicator of negative welfare (e.g., self-harming behavior) requires immediate action, but there is no reason to identify or address one type of indicator before the other; they can be assessed and addressed simultaneously. Extending the consideration of positive versus negative welfare states to an animal's entire life, Yeates (2011) discusses the concept of a "life worth living," which in general terms refers to an animal's life in which positive experiences outweigh negative experiences in terms of frequency, duration, or degree. This is contrasted with a "life not worth living" in which negative experience outweighs positive experience (Yeates, 2011). While there are limitations with the implementation of this concept at a broad scale (Yeates, 2011), adopting a philosophy of providing animals with as much positive experience as possible while minimizing negative experiences when feasible is consistent with ethical use of animals.

Animal-Based Assessment of Welfare

The fourth development in animal welfare science, and specifically in welfare assessment, involved expanding the focus of assessment from purely resource-based measures, such as the quality of housing or diet, to include animal-based measures, such as wounding rates or behavior (Hewson, 2003; Leach et al., 2008; Webster, 2005b; Webster et al., 2004; Whay, 2007). This change reflected a widespread acknowledgment that the most relevant assessments of animal welfare, indeed by definition (Gonyou, 1993), are those that focus on the animal and how it is faring. Studies of farm animals demonstrate that animal-based measures of animal welfare can vary dramatically across facilities (Fraser, 2014; Main et al., 2003), even among farms that employ the same housing systems or resource-based welfare certification programs, and that performance on certain welfare indicators does not necessarily correlate with participation in a certification program (Main et al., 2003). Given the limited predictive value of resource-based welfare management and assessment, it has been suggested that welfare issues at individual sites would be better addressed at the local level rather than modifying overarching guidance, legislation, or certification programs (Main et al., 2003).

The growing understanding of the need for animal-based assessment measures is particularly relevant to considerations regarding the welfare of laboratory animals, including dogs, for whom current requirements governing use, care, and housing (e.g., *Guide for the Care and Use of Laboratory Animals* [the *Guide*; NRC, 2011]) emphasize resource-based indicators, such as parameters of diet or housing, over animal-based indicators. Nonetheless, animal-based measures of welfare have been developed for laboratory animals, including mice (e.g., Leach et al., 2008; reviewed in Beaver and Bayne, 2014). A number of welfare assessment tools have been developed for dogs, including those with spinal cord injuries (e.g., Budke et al., 2008), cardiac disease (e.g., Freeman et al., 2005), and chronic pain (Wiseman-Orr et al., 2004, 2006), as well as for healthy dogs (e.g., Lavan, 2013), with a recent review by Belshaw et al. (2015). Comprehensive welfare assessments that include animal-based measures are now mandated by the Association of Zoos & Aquariums (AZA, n.d.), a professional accrediting body in the zoological profession.

Conclusion 5-1: Animal welfare is multi-dimensional, reflecting the health, comfort, behavior, and emotions of animals in human care. There is a need for the U.S. Department of Veterans Affairs to combine aspects of each of the three major approaches to animal welfare, explicitly consider positive and negative welfare states, and measure animal-based welfare indicators in order to enhance the positive welfare of laboratory dogs.

ADDITIONAL CONSIDERATIONS FOR ENHANCEMENT OF THE WELFARE OF LABORATORY DOGS

Considerations Related to Human and Conspecific Contact

Several studies suggest that the presence of human companions may be more effective in reducing behavioral and physiological indicators of stress in dogs than the presence of a canine companion (Pettijohn et al., 1977; Tuber et al., 1996). Specifically, physical contact with humans in the form of stroking or handling has been demonstrated to have a positive influence on a dog's behavior and physiological reactions to stress (Fuller, 1967; Hennessy et al., 1998; Lynch and McCarthy, 1967; also see review by Payne et al., 2015). Slow, firm stroking after one venipuncture was found to decrease subsequent cortisol production after a second venipuncture 20 minutes later (Hennessy et al., 1997, 1998). These findings led Tuber et al. (1999) to conclude that even single instances of human interaction with familiar or unfamiliar people can have an ameliorating influence on dogs in shelter environments. Payne et al. (2015) review literature on dog–human bonding that demonstrates that certain human behaviors (e.g., affiliation and attention) contribute to positive emotional states in dogs, and stronger bonds between dogs and humans are associated with lower physiological arousal in dogs, as measured by cortisol levels.

Experimental and observational research indicates that many dogs benefit from contact with other dogs as well as with humans (see Meunier and Beaver, 2014; Taylor and Mills, 2007, for reviews). For example, dog activity increased when human caretakers were actively working in a housing facility and decreased during lunch hours and overnight, although an additional 30 minutes of human contact did not appreciably increase activity (Hughes and Campbell, 1990). (The authors of this study were unable to distinguish between normal locomotor patterns and repetitive, possibly abnormal movements.)

A study in shelter dogs showed that the most important variable contributing to the level of welfare of dogs was the opportunity to regularly leave the cage for a walk, whereas other variables like gender, cage size, individual housing, and reproductive intactness had no significant effect on the physiological indicators of welfare. Dogs that had the opportunity to go on walks had high antioxidant capacities and performed displacing activities or stereotyped behaviors less frequently (Cafazzo et al., 2014). Dogs that were simply given a larger cage to exercise in as opposed to a walk had total antioxidant capacity similar to dogs that were not put into larger exercise cages (Cafazzo et al., 2014).

Clark et al. (1997) found that placing individual dogs in out-of-cage exercise areas for 20 minutes several days per week did not increase activity or prevent the development of abnormal behaviors. While there is no strong evidence that cage size has an effect on activity, many of the existing studies compared very small cages to slightly larger cages, which may not be saliently different to the dogs, and these studies involved small sample sizes (Taylor and Mills, 2007). While enclosure size may be less important than the quality of the space and opportunities for socialization, enclosures containing multiple animals must be large enough for the animals to rest separately, to make use of visual barriers between themselves and cage mates, and to sleep and eat away from areas used for defecation and urination (Meunier and Beaver, 2014). Hubrecht and Buckwell (2007) go so far as to recommend that laboratory environments enable dogs to be housed

in social groups and that solitary housing be avoided whenever possible and only implemented in exceptional circumstances. U.S. Department of Agriculture (USDA) regulations do not require social housing, but if the animals are group housed, they have to be compatible.[1]

Considerations Related to Comfortable Environments

Many texts and overviews exist that provide guidance on optimal care, housing, and the management of dogs in laboratory environments in order to optimize welfare (e.g., Hubrecht and Buckwell, 2007; Meunier and Beaver, 2014; NRC, 1994; Prescott et al., 2004; Stafford, 2006). Tuber et al. (1999) describe the importance of having a simulated "living room" in a dog shelter, which has the added benefit of acclimating dogs to home-like environments. For laboratory dogs, this would correspond to having a place apart from the kennel environment and other dogs where humans and dogs can interact in a quieter setting. Dogs may vary, according to breed and disposition, in their requirement for this type of interaction and setting, but if it is made available, then individual dogs' responses to such an environment can be evaluated through an animal-based assessment.

Access to enrichment toys is important for visual, olfactory, and social stimulation. A frequent rotation of enrichment is recommended, as dogs can lose interest in a given item within 1 or 2 days (Overall and Dyer, 2005; Wells, 2004).

Relationship of Current Standards, Requirements, and Recommendations to Developments in Animal Welfare Science

Legislation, policies, and regulations pertaining to the welfare of dogs in research were summarized in Chapter 2. While these various regulatory and oversight processes provide a wide-ranging framework for animal care and use with assessments mandated by regulation, their focus is almost exclusively input-based (concerned with practices and features provided to the animals). Current USDA regulations require daily observation of animals to assess their health and well-being; additionally, institutional animal care and use committees (IACUCs) are required to inspect all animal facilities and study areas every 6 months to review the condition of the animals.

Conclusion 5-2: While the Guide for the Care and Use of Laboratory Animals *and the Animal Welfare Act provide a foundation for the assessment of well-being, the U.S. Department of Veterans Affairs has an opportunity to incorporate current developments in animal welfare science into its animal care and use program.*

Clarification of AAALAC International Accreditation and Use of the *Guide* as Regulations

In addition to the general provisions in the *Guide* mentioned in Chapter 2, the *Guide* discusses several specific areas of attention pertinent to the welfare of dogs during routine care and in experimental training. The *Guide* notes that enclosures that provide greater freedom of movement and vertical height (e.g., kennels) are preferred for dogs, and if they are housed individually or in smaller enclosures, dogs should be given the opportunity to socialize and experience positive human interaction (NRC, 2011). Additional opportunities for socialization mentioned include being walked on a leash, having access to a run, or being moved into areas for play, exploration, and social contact (NRC, 2011). Manipulatable toys are useful for enrichment and should be rotated to maintain

[1] CFR Title 9, Subchapter A: Animal Welfare; see §§ 3.7, 3.8. Available at https://www.govinfo.gov/content/pkg/CFR-2013-title9-vol1/pdf/CFR-2013-title9-vol1-chapI-subchapA.pdf (accessed March 24, 2020).

the animal's interest (NRC, 2011). The *Guide* encourages the use of behavioral conditioning to habituate and train animals to participate voluntarily in experimental procedures.

The comprehensive peer-review process conducted by AAALAC International encompasses the review and assessment of the effectiveness of implementation of the elements noted above in programs of laboratory research with dogs. AAALAC International site visits normally occur triennially; however, due to the public interest in and visibility of research with dogs at the VA, visits focused on the care and use of dogs at the VA are now conducted by AAALAC International every 18 months. The AAALAC International accreditation process identifies programmatic strengths and weaknesses according to the *Guide* and engages the institution to pursue timely corrective actions of problematic areas. Moreover, the professional interactions of the AAALAC International site visit process incorporate two other important principles in ethics and in oversight from the *Guide*. First, programmatic leadership is reminded that the use of animals in research is a privilege and that "[e]thical considerations discussed here and in other sections of the *Guide* should serve as a starting point; readers are encouraged to go beyond these provisions" (NRC, 2011, p. 4). Second, as a practical and operational matter, "[t]he body of literature related to animal science and use of animals is constantly evolving, requiring Programs to remain current with the information and best practices" (NRC, 2011, pp. 12–13).

Relevant International Guidelines or AAALAC International and Non–U.S. Department of Agriculture Recommendations and Standards with Regard to Dogs

While the VA is only required to follow U.S. regulations, there may be value in consulting regulations from other countries to determine if enhancements to existing programs could be made. Considering the committee's Statement of Task and the directive in Chapter 1 of the *Guide* noted above, it is useful to examine approaches identified in other international regulatory frameworks that are intended to enhance the welfare of dogs used in research while permitting the attainment of research objectives. The countries of the European Union are expected to follow Directive 2010/63EU (EUR-Lex, n.d.) on the protection of animals used for scientific purposes through the transposition of the principles of the directive into national regulations. It is beyond the scope of this discussion to summarize each of the national regulations spawned by Directive 2010/63EU in order to identify elements that would potentially improve laboratory dog animal welfare. However, the implementation details of Directive 2010/63EU elaborated in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (ETS 123) (Council of Europe, n.d.) provide some insight. The principles in ETS 123 are largely consonant with those of the *Guide* but offer specific provisions that may confer additional animal welfare benefits to laboratory dogs. For example, ETS 123 emphasizes that dogs may not be singly housed for more than 4 hours, and if for experimental purposes they must be singly housed for longer than that, then they should, if possible, be afforded time daily for human socialization and visual, auditory, and tactile contact with other dogs (Council of Europe, n.d.). Also, the space requirements for laboratory dog housing in ETS 123 afford dogs more space for all weight categories than the dog housing space recommendations of the *Guide*. In addition to specifying more space, the ETS stipulates that no less than 50 percent of the space will be dedicated to the indoor housing area and that an outdoor run area should be provided where possible (Council of Europe, n.d.). It is widely recognized that the mere availability of more space does not ensure that the space will stimulate increased exercise or beneficially enhance the environment of the enclosure for the dog; the quality of the space provided must also be recognized as an essential parameter (Campbell et al., 1988; Hite et al., 1977; Hughes et al., 1989). The Australian *Guidelines for the Care and Housing of Dogs in Scientific Institutions* (NSW Agriculture, 1999), citing the work of Serpell and others (Serpell, 1995), provides a detailed discussion of the design of the dog housing enclosure, emphasizing its important

role in dog social interactions, environmental control/stimulation, comfort, and exercise. The UK National Centre for the Replacement Refinement & Reduction of Animals in Research provides comprehensive information on laboratory dog housing, tools for assessing the welfare of laboratory dogs, and opportunities for refining procedures in studies (NC3Rs, n.d.).

Conclusion 5-3: In the pursuit of enhancements to the care and use of laboratory dogs in U.S. Department of Veterans Affairs (VA) research, international regulations and guidelines may be a useful resource for alternate approaches and recommendations. International guidelines and recommendations offer the VA programmatic leaders and institutional animal care and use committees expanded perspective on the contemporary literature and trends on laboratory dog care.

ASSESSMENT OF CURRENT VA PRACTICES: DO THEY MEET CURRENT STANDARDS, REQUIREMENTS, AND RECOMMENDATIONS WITH REGARD TO THE CARE AND WELFARE OF DOGS?

Members of the committee conducted site visits to two VA programs engaged in dog research—one at the McGuire VA Medical Center (VAMC) Veterinary Medical Unit in Richmond, Virginia, and the other at Washington University School of Medicine in St. Louis, Missouri. The purpose of these visits was to meet with the personnel involved in the oversight, care, and research on dogs and to observe the conditions and practices associated with this use. Materials obtained in advance of the site visit to the Richmond VAMC[2] indicated that the practices relating to dog husbandry and care were sound and conformed with the requirements specified in the Animal Welfare Act (AWA)/Animal Welfare Regulations (AWR)[3] and the *Guide* (NRC, 2011). These materials included two letters of accreditation from AAALAC International, which strongly supported the VA's assertion of a high-quality program for the care and use of dogs; the 2019 AAALAC International letter described the VA program as "exemplary." An external review by the National Institutes of Health's Office of Laboratory Animal Welfare (OLAW, 2019) found provisions for the care of dogs at the Richmond VAMC to be in compliance, as did upper management within the VA system.

Strong administrative support for the Richmond VA dog research program was evident. Specifically, two new positions had recently been added, augmenting the resources available for dog care and use in the areas of surgery, post-procedural care, behavioral management, and socialization. These enhancements were buttressed by a new electronic clinical veterinary record system with which to document care. The administration also supported ample training and educational opportunities for the animal facility staff.

A large body of research with livestock has demonstrated that general attitudes toward animals, attitudes about aspects of working with animals, and the behavior of stockpersons toward animals (specifically negative and positive interactions) have measurable impacts on the behavior and physiology—and thus the welfare—of the animals that these individuals care for. Such impacts extend to production metrics (e.g., milk yields, farrowing rates, egg production) in a variety of species (Hemsworth and Coleman, 2011). Research also demonstrates that human companion or caretaker attitudes and behavior correlate with welfare indicators in dogs (Payne et al., 2015) and in non-domesticated species maintained in zoos (e.g., Carlstead et al., 2019; Wielebnowski et al., 2002; see review by Cole and Fraser, 2018). Mellen (1991) observed that positive interactions with caretakers resulted in greater reproductive success in non-domestic, zoo-housed felids. Positive

[2] VA (U.S. Department of Veterans Affairs). 2019. McGuire VAMC Richmond NAS response, July 2019, and AAALAC International letter, 2019, to inform committee subgroup site visit.

[3] CFR Title 9, Subchapter A: Animal welfare. Available at https://www.govinfo.gov/content/pkg/CFR-2013-title9-vol1/pdf/CFR-2013-title9-vol1-chapI-subchapA.pdf (accessed March 24, 2020).

interactions with humans are also associated with positive impacts on laboratory species (e.g., Bayne, 2002; Bloomsmith et al., 1997; Waitt et al., 2002). Positive attitudes about animals are correlated with job enjoyment and willingness to learn in stockpeople (Coleman et al., 1998), whereas lower caretaker job satisfaction can be associated with a weaker human–animal bond (Carlstead et al., 2019). The strength of the human–animal relationship is reinforced through positive caretaker behaviors toward the animals and animal responses to the caretaker; the relationship is reciprocal (Hemsworth, 2003).

This is relevant because during site visits at both the Richmond and St. Louis facilities, several observations surfaced that would suggest that human–animal relationships involving the laboratory dogs are positive, provide mutual benefits to animal and caretaker, and are reinforced via job satisfaction. First, at both facilities the laboratory dogs enthusiastically approached and engaged with caretakers and laboratory personnel, suggesting that the dogs viewed this contact positively, which would not be expected if the dogs were fearful of these individuals. Second, the staff reported high dedication to their jobs and commitment to the animals' well-being. Associated with this, staff at both facilities reported having good access to resources, financial and otherwise, for enriching the dogs' lives. Third, staff reported at both facilities that, despite their support for animal research, often the most difficult or least favorite parts of their jobs were managing their attachments to the animals, knowing the animals would be euthanized at the end of the study. Staff got to know each dog individually and developed a relationship with the dog, and this was at times difficult to manage when studies ended. Fourth, staff reported having excellent support for continuing education to maintain and advance their skills in animal care and saw opportunities for professional growth in their organizations. Finally, staff were knowledgeable and comfortable with the processes in place for bringing up concerns about animal care and welfare. Procedures were available for reporting animal welfare concerns anonymously, if the staff person so chose.

Through discussion with IACUC members, researchers, and veterinary staff, it was determined that the IACUCs were functioning well within the guidelines of the VA and federal regulations. In St. Louis, there appeared to be good communication between the Washington University IACUC and the VA IACUC. Both the Richmond and St. Louis IACUCs approached harm–benefit analysis by discussing concerns and weighing potential harms and benefits in a deliberative process. The IACUCs described well-defined procedures and practices for reporting unusual findings or adverse events in the laboratory dogs and ensuring prompt and appropriate engagement of the veterinary medical officer to manage clinical events arising either spontaneously or due to experimental complications. At Richmond, veterinary care was under the direction of an external consultant who had appropriate training, experience, and involvement in all relevant areas of the program and good rapport with research and animal care staff. At St. Louis, veterinary care was provided by the onsite veterinarian and his staff. The process for selection and approval of the dog model was reaffirmed in meetings that committee members had with the investigators conducting dog research and with the IACUC. Interestingly, the VA had also provided funding for studies approved by the IACUC to enable the investigators to explore the experimental feasibility of using pigs as an alternative to dogs.

Dogs at both facilities were individually housed two to five per room in primary enclosures (pens) that met the federal space requirements and afforded the animals an opportunity to exercise. In Richmond, the pens were constructed of galvanized wire separators and did not contain rest boards or other structural embellishments. Padded beds were provided to some dogs when available. In St. Louis the pens were the size required by the AWR for exercise opportunity for individually housed dogs. The runs had stainless steel partitions with chain link tops that allowed the dogs to see over the partition. The fronts of the cages were chain link so a dog could view the dog across from it in the room. There were only two dogs present in St. Louis. One was male and one was female, so they were separately housed, but they had visual contact with each other. Each run had

a resting board. An enrichment toy was present in each cage and changed at least weekly or as needed for sanitation.

At Richmond, the dogs were housed in individual pens due to constraints of the data collection technology. The dogs had surgically implanted sensors for collecting data on cardiac function. A portion of the sensor protruded from the dog's ventrolateral thorax, raising concerns that dog–dog interactions could damage the sensor or injure the dog. Additionally, the data collection from the sensors is remote and continuous, and two sensors in very close proximity interfere with one another's signals.

In Richmond, groups of dogs were released into a communal exercise enclosure for approximately 45 minutes 5 days per week for additional supervised socialization with technicians, who encouraged dog–dog interactions and play with enrichment objects. Various enrichment devices were rotated through the dogs' primary enclosures to maintain their interest, and a special food item was provided weekly. On weekends, dogs were released from their home cages for 10 minutes daily during the cage cleaning procedure. Sporadically, dogs were permitted to freely range or walk on leash more widely to the office areas of the animal facility with supervision. In St. Louis, the dogs were released into a hallway at least twice per week with human interaction. They could freely run the hall to chase balls or interact with people. Dogs were also released within the room while caretakers were cleaning runs. The staff at the St. Louis site is planning to obtain pen partitions that would allow the dogs to move between runs for more space. They are also considering a separate playroom to use rather than the current hallway.

Both the Richmond and St. Louis support facilities, such as surgeries and treatment rooms, were state of the art and well maintained.

Conclusion 5-4: Based on the information obtained during site visits and in materials submitted to the committee, including AAALAC International accreditation letters and the Office of Laboratory Animal Welfare report on assessment of the U.S. Department of Veterans Affairs (VA) canine research program, the committee concludes that the VA appeared to meet or exceed current regulatory requirements. Nonetheless, the committee observed several areas where the VA's animal program could be enhanced, and those enhancements are included in the recommendations below.

CONSIDERATIONS FOR ENHANCEMENT AT THE VA

Recommendation 6: Enhance the welfare of laboratory dogs used for biomedical research.

The U.S. Department of Veterans Affairs (VA) should enhance the welfare of laboratory dogs used in biomedical research in their facilities in the following ways:

- **Submit to voluntary U.S. Department of Agriculture (USDA) inspections of dog facilities to increase transparency.**
  - **As a federal agency, the VA must meet all Animal Welfare Act requirements, but it is not subject to mandatory inspection by the USDA. Requesting voluntary USDA inspections of dog facilities would not only increase transparency (as summaries of results from USDA inspection reports are posted on a public website), but it would also provide another independent peer review of the VA's animal care practices and facility compliance from a neutral, independent third party.**
- **Modify dog enclosures and staffing to enhance opportunities for social interaction, exercise, and sensory stimulation.**

- **Ensure staffing is to a level sufficient for all dogs to have 30–45 minutes outside of their primary enclosures 7 days per week.**
- **Deploy a system of adjoining cages with barriers or transfer doors. When the facility is not fully occupied, this type of system would provide the dogs with more space, more behavioral choices, and more opportunities for exercise and could enable compatible dogs to have tactile contact.**
- **To the extent compatible with the needs of studies, maximize the amount of time dogs are able to interact with humans or be let out of their primary enclosures.**
- **When it is compatible with study goals and safe for the dogs and personnel, create an outdoor space for laboratory dogs to visit on a regularly scheduled basis. This would provide additional opportunity for exercise as well as olfactory, sensory, and visual stimulation; a variety of experiences; and time with humans.**
- **Increase the amount of enrichment available to dogs, and continue to evaluate and incorporate new options for environmental enrichment, including olfactory enrichment, on a regularly scheduled basis.**

- **Address current experimental impediments to dog–dog interactions.**
  - **Given concerns about possible wounding or damage during the social housing of dogs wearing internal or external (implanted) devices, fund a pilot study at the end of an existing protocol to examine the risk of these outcomes.**
  - **Consider an alternative placement of implanted devices to decrease the likelihood of complications from socialization with other dogs.**
  - **Encourage the development and use of miniaturized devices that are less cumbersome for the animals and less likely to be damaged, especially if they can be implanted subcutaneously.**
- **Conduct enhanced assessments of laboratory dog welfare.**
  - **To move beyond simple observations of dogs' health, VA staff involved in the care and welfare of laboratory dogs should collaborate on continuous education and continuous improvement of measures that advance laboratory dog welfare.**
  - **VA veterinary and animal care staff, facilities personnel, members of the institutional animal care and use committee, and principal investigators should conduct formal, written assessments of animal welfare that reflect the state of the art in animal welfare assessment methods.**

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Appendix A

Methods

This appendix includes public meeting agendas, a list of materials supplied to the committee by the U.S. Department of Veterans Affairs (VA), and the literature search criteria used by the committee. The information-gathering sessions included in-person, public meetings and webinars held by the committee from December 2018 to May 2019, and they are listed in chronological order.

PUBLIC MEETING AGENDAS

Meeting 1
Sunday, December 9, 2018
National Academy of Sciences Building, Washington, DC

1:00 p.m.	**Welcome, Introductions, Process for Open Session** – Rhonda Cornum, Committee Chair
1:10 p.m.	**The National Academies of Sciences, Engineering, and Medicine Study Process Overview** – Lida Anestidou, Study Director
1:20 p.m.	**Study Context and Expectations for the Study** – Michael Fallon, U.S. Department of Veterans Affairs – Alice Huang, U.S. Department of Veterans Affairs – Joan Richerson, U.S. Department of Veterans Affairs
3:00 p.m.	**Public Comment Session**
3:20 p.m.	**Closing Remarks and Adjournment of Open Session**

Meeting 2
Thursday, February 14, 2019
National Academies Keck Center, Washington, DC

8:30 a.m. **Welcome, Introductions, Process for Open Session**
– Rhonda Cornum, Committee Chair

8:45 a.m. **Study Context and Expectations for the Study**
– Michael Fallon, U.S. Department of Veterans Affairs
– Alice Huang, U.S. Department of Veterans Affairs (remote)
– Joan Richerson, U.S. Department of Veterans Affairs (remote)

10:15 a.m. **Public Comment Session**

10:30 a.m. **Closing Remarks and Adjournment of Open Session**

Meeting 3 and Workshop on the Uses of Dogs in Biomedical Research
Wednesday, March 27, 2019
National Academies Keck Center, Washington, DC

8:00 a.m. **Welcome, Introductions, Process for Open Session**
– Rhonda Cornum, Committee Chair

Panel on Comparative Modeling in Cardiovascular Research

Cardiovascular research is a key priority for the VA and one of the areas in which dogs serve as models of disease. This panel will examine various approaches to modeling diseases of the heart for research and translational purposes.

8:10 a.m. **Panel Introduction**
– Donna K. Arnett, Committee Member

8:15 a.m. **Modeling Cardiovascular Disease Using Canine Models**
– Rodney White, Committee Member, Long Beach MemorialCare Heart & Vascular Institute

8:35 a.m. **Translational Approaches in Cardiovascular Disease Research Using Rodent Models**
– David Harrison, Vanderbilt University Medical Center

8:55 a.m. **Modeling Cardiovascular Disease Using Swine Models**
– Daniel D. Myers, University of Michigan

9:15 a.m. **Break**

9:25 a.m. **Using Human Hearts to Study Arrhythmogenesis**
– Igor Efimov, The George Washington University

9:45 a.m. **CiPA: Evaluating Risk Using Mechanistic Electrophysiologic Understanding of Proarrhythmia**
– Alexandre Ribeiro, U.S. Food and Drug Administration

10:05 a.m. **Cardiovascular Research in Humans: The Clinician's Perspective**
– Scott A. Bernstein, NYU Langone Health

10:25 a.m. **Discussion with Panelists**

10:55 a.m. **Break**

Panel on Ethical and Societal Issues Regarding the Use of Dogs in Biomedical Research

11:10 a.m. **Panel Introduction**
– Margaret (Mimi) Foster Riley, Committee Member

11:15 a.m. **Establishing a Necessity-Based Approach to the Use of Chimpanzees in Research**
– Jeffrey Kahn, Johns Hopkins University

12:00 p.m. **Lunch**

1:00 p.m. **An Ethical Framework for the Use of Animals in Research**
– David DeGrazia, The George Washington University

1:30 p.m. **The Unique Role of Dogs in Society**
– James A. Serpell, University of Pennsylvania

2:00 p.m. **Discussion with Panelists**

2:30 p.m. **Break**

Panel on Comparative Modeling in Spinal Cord Injury Research

Spinal cord injury (SCI) research is a key priority for the VA and one of the areas in which dogs serve as models of disease. This panel will examine various approaches to modeling SCI for research and translational purposes.

2:45 p.m. **Panel Introduction**
– Warren Casey, Committee Member

2:50 p.m. **An Overview of Large Animal Models of Spinal Cord Injury**
– Candace L. Floyd, University of Utah Health

3:10 p.m. **Rodent Models of Spinal Cord Injury**
– Warren J. Alilain, University of Kentucky College of Medicine

3:30 p.m. **The Natural SCI Model of Canine Intervertebral Disk Herniation Clinical Trials of Novel Therapies**
– Nicholas Jeffery, Texas A&M College of Veterinary Medicine

3:50 p.m.	**The Natural SCI Model of Canine Intervertebral Disk Herniation Clinical Trials of Novel Therapies (Continued)**
	CANSORT-SCI and the International Canine SCI Registry: Tools for Identifying and Assessing the Impact of Therapeutic Strategies – Sarah Moore, The Ohio State University College of Veterinary Medicine
4:10 p.m.	**Discussion with Panelists**
4:40 p.m.	**Public Comments**
4:55 p.m.	**Adjourn Open Session**

Meeting 4
Thursday, March 28, 2019
National Academies Keck Center, Washington, DC

8:30 a.m.	**Welcome, Introductions, Process for Open Session** – Rhonda Cornum, Committee Chair
8:40 a.m.	**Establishing Research Priorities at the VA** – Rachel Ramoni, Veterans Health Administration – Karen Lohmann Siegel, Veterans Health Administration – Chris Bever, Veterans Health Administration
9:30 a.m.	**Adjourn Open Session**

Webinar 1
Tuesday, May 7, 2019

9:30 a.m.	**Welcome, Introductions, Process for Webinar** – Rhonda Cornum, Committee Chair
9:40 a.m.	**Panel Introduction** – Nancy Figler Marks, Committee Member
9:45 a.m.	**Spinal Cord Injury Research in Humans: The Clinician's Perspective** – James Guest, University of Miami
10:05 a.m.	**Modeling Infectious Disease Research Using Canine Models** – Christine Petersen, The University of Iowa
10:25 a.m.	**Committee Discussion with Panelists**
11:00 a.m.	**Adjourn Webinar**

Webinar 2
Tuesday, May 28, 2019

10:30 a.m.	**Welcome, Introductions, Process for Webinar** – Greg Symmes, Executive Director, Division on Earth and Life Studies, National Academies of Sciences, Engineering, and Medicine
10:35 a.m.	**Panel Introduction** – Warren Casey, Committee Member
10:40 a.m.	**Development of Human-Based Computer Model of the Heart to Predict Drug Safety and Efficacy** – Elisa Passini, Senior Researcher, Department of Computer Science, University of Oxford
11:00 a.m.	**Modeling Atrial Fibrillation Using Human Embryonic Stem Cell–Derived Atrial Tissue** – Zachary Laksman, Director, St. Paul's Hospital Atrial Fibrillation Clinic and Director, Inherited Arrhythmia Clinic, University of British Columbia, Vancouver, Canada
11:20 a.m.	**Committee Discussion with Panelists**
12:00 p.m.	**Adjourn Webinar**

OVERVIEW OF DOCUMENTS PROVIDED BY THE VA

The documents below were provided or submitted by the VA to the committee during the course of the study. Copies of the documents can either be found at the VA website[1] or are deposited in the study's public access file.[2]

- **Overview of VA animal research and canine studies[1]**
  Includes VA research overview, organizational structure, collaborative relationships, research regulatory environment, animal research conduct and review, and past and current research using dogs.
- **Appendix 1: Veterans Health Administration (VHA) handbook 1200.07, *Use of Animals in Research*[2]**
  Includes the rationale for and principles governing use of animals in research, responsibilities of the Chief Veterinary Medical Officer (CVMO) at the VA central office, organization at VA medical facilities, veterinary medical unit operations at VA medical facilities, institutional animal care and use committees (IACUCs), visits to VA animal facilities by non-VA federal regulators, and requirements for occupational health and safety program.
- **Appendix 2: VHA handbook 1058.01, *Research Compliance Reporting Requirements*[2]**
  Includes a summary of major changes to reporting requirements; systemic requirements and responsibilities; reporting guidance for death (unanticipated or related to the research) in human research; reporting guidance for unanticipated animal death, theft, escape, or disappearance in animal research and death/accident/illness/injury/exposure of humans working with animals; and reporting guidance on incidents related to research safety, research laboratory security, and research information security.
- **Appendix 3: Animal component of research protocol (ACORP), version 4[2]**
  Copy of the ACORP, a form that contains the justification for proposed animal research at the VA. The ACORP is used by the local IACUC to assess harm–benefit.
- **Appendix 4: VA semiannual evaluation of the institutional animal care and use program and facilities[2]**
  Copy of the form that the VA requires for the semi-annual review of all of the policies, plans, standard procedures, and systems for ensuring humane animal care and use. The form has three parts: Part 1—Checklist; Part 2—Table of Deficiencies and Departures; Part 3—Post-Review Documentation.
- **Appendix 5: Adoption of research animals covered by the Animal Welfare Act regulations (Guidance Document AR2018-001)[2]**
  Guidance document used to assist VA animal research programs in arranging adoptions for laboratory animals as pets.
- **Appendix 6: Selected VA research accomplishments with dogs[1]**
  Information on past projects that used dogs to address veterans' health issues from 1960 to the current decade.
- **Appendix 7: *Canine, Feline, and Non-Human Primate Research Protocols* (Guidance Document: AR2017-001, rev. 2)[2]**
  Information on the reviews required for research protocols involving dogs, cats, and non-human primates that are conducted at any VA property.

[1] See https://www.research.va.gov/programs/animal_research/canine_research/nas_assessment.cfm (accessed June 16, 2020).

[2] Copies of documents in the public access file may be requested by contacting the National Academies' Public Access Records Office (PARO@nas.edu).

- **Appendix 8: Current VA research using canines**[1]
  Information about research protocols as of June 1, 2017; March 28, 2018; and November 15, 2018: animal protocol form approved by the local IACUC and the CVMO's office; feedback document from the CVMO's office used by the local IACUC to develop the final approved version of the animal protocol form; and a summary of the literature search done by the CVMO's office as part of the review.
- **Appendix 9: Disclosure of animal research documents pursuant to FOIA request**
- **Completed ACORP for a companion dog study**[2]
  This study became active in 2019; this was the first protocol approved through the new review process (AR-2017).
- **Publications related to Appendix 8**[1]
  A list of peer-reviewed publications related to each of the protocols in Appendix 8 (current VA dog projects).
- **Appendix 9: Veterans Health Administration Freedom of Information Act (FOIA) guidance on animal research requests**[1]
  Memo from the VHA FOIA office to VHA field FOIA officers on how to respond to FOIA requests for information on VA animal research.
- **Canine research in the Department of Veterans Affairs** (PowerPoint presentation)[1]
- **Publications from the past 10 years stemming from VA research with dogs**[1]
  List of all peer-reviewed, full-length reports of research with dogs that indicated author affiliation with a VA facility, support by VA funding, or other use of VA resources.
- **VA response to follow-up questions of the National Academies committee**[1]
  The committee asked for clarification on the information provided by the VA. The committee was interested in connections and relationships between past projects, current projects, and accomplishments. This document contains the VA's response to the committee.
- **Report on the National Institutes of Health Office of Laboratory Animal Welfare (NIH-OLAW) site visits to the Veterans Affairs medical centers with focus on canine care and use in research** (January 3, 2019).
- **Grant applications of four active protocols**[2]
  - Protocol 1 (High-frequency spinal cord stimulation to restore cough)
  - Protocol 5 (Mechanistic insight of premature bentricular contractions-induced cardiomyopathy)
  - Protocol 6 (Autonomic nerve activity and cardiac arrhythmias)
  - Protocol 7 (Effect of chronic premature ventricular contractions on the remodeled ischemic heart)

LITERATURE SEARCH CRITERIA (CHAPTER 3)

A literature search, which was performed using the Scopus database, employed the following approach: "dog" and "cardi" were the standard spine, and modifiers were then added. The modifiers and numbers of citations obtained were as follows: arrhythmia (219), atrial fibrillation (144), aneurysm (19), congestive heart failure (34), ventricular premature contraction (6), device (95), gene therapy (10), graft (49), instrument (52), pacemaker (37), stent (169), surgical technique (77), thrombosis (21), tissue engineering (3), and vascular disease (120). Many papers were duplicated among the search subsections. A subsequent PubMed search turned up additional articles. A subset of the articles obtained from both searches is referenced in this section of the report, with an effort to cite only papers that describe dog use in hypothesis-testing research or proof-of-principle applications.

To gain an understanding of dog use in pharmacological research over the past decade, a literature search was performed using Scopus and PubMed databases in April 2019 to identify published scientific literature (2009–2019) having the words "dog" and "pharmacol" in the title or abstract. Citations were characterized as either (1) basic research or preclinical research/development of human therapeutics; (2) veterinary research/product development; (3) companion/therapeutic animal research; or (4) other, unrelated to any of the above.

Appendix B

U.S. Department of Agriculture Statistics on the Use of Dogs and Other Animals in Research

The Animal Welfare Act of 1966[1] (AWA) mandated that the U.S. Department of Agriculture (USDA) collect annual reports listing the numbers of vertebrate animals (excepting mice, rats, and birds) used by all USDA-registered academic and industrial research facilities, as well as federal research facilities,[2] including the U.S. Department of Veterans Affairs (VA). Reports from individual facilities to USDA's Animal and Plant Health Inspection Service (APHIS) are publicly available (USDA AHPIS, n.d.a), as are annual summaries of regulated animal use by state (USDA APHIS, 2020). For USDA purposes, "research" includes teaching, testing, and research; hence, these numbers include all AWA-regulated species used by academic, industrial (public or private), and government research facilities engaged in professional training, biomedical research, or product development/safety testing while excluding animal breeding and other activities related to the pet industry. The USDA data also include animals that were bred for research but have not yet been used. Hence, the USDA statistics provide the most complete and detailed view of trends in total and individual species usage for research (excluding mice, rats, and birds) within the U.S. research enterprise over the past 45 years. Using data from the USDA website (USDA APHIS, 2020) for the years 2008–2018 and from archived annual reports (USDA APHIS, n.d.b) for prior years, the committee investigated trends in the use of dogs and other regulated species from 1973 to 2018, the results of which are summarized in Figure B-1.

The USDA data on total regulated animal use reveal the following trends over the past 45 years:

[1] U.S. Code Title 7, Chapter 54: Transportation, sale, and handling of certain animals. Available at https://www.nal.usda.gov/awic/animal-welfare-act (accessed December 30, 2019).

[2] Federal research facilities are exempt from USDA registration but required to file the same animal use reports as registered facilities.

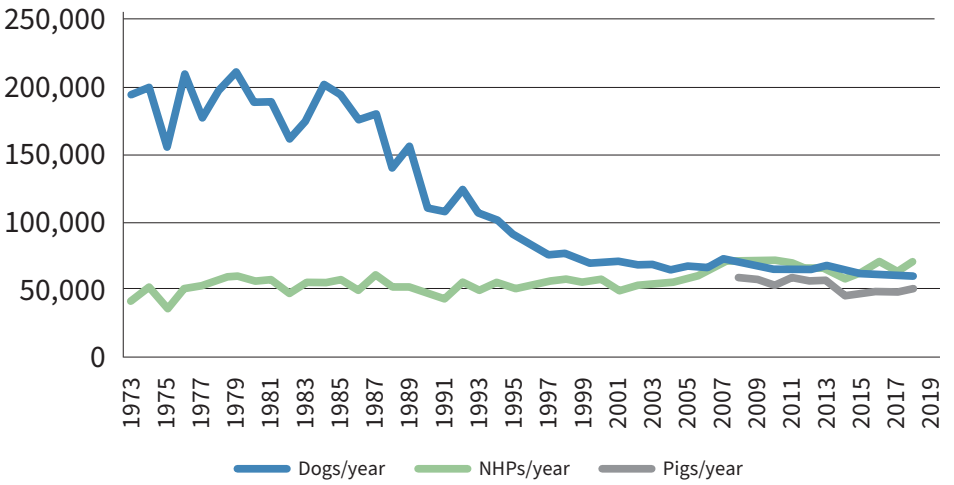

FIGURE B-1 Annual dog, non-human primate (NHP), and pig usage 1973–2018 in the United States, based on data collected by the U.S. Department of Agriculture.
SOURCES: For 2008–2018, USDA APHIS (2020); for 1973–2007, USDA APHIS (n.d.b).

1. There was a substantial decrease in dog use from 1973 to 2018 (see Figure B-1). Dog use peaked at more than 200,000 per year in the late 1970s to early 1980s and then entered a 20-year period of decline. By the early 21st century, dog usage plateaued at roughly 60,000 per year, representing a 65 percent decrease from prior peak levels. This level of annual dog use has remained relatively stable over the past two decades.
2. Within the same time period, use of cats, guinea pigs, hamsters, rabbits (1973–2007), and farm animals (1990–2007) also decreased by 50 percent or more (data not shown).
3. Indeed, the only categories to increase over this period were non-human primates (NHPs) and "other covered species."
4. Total reported animal usage among all AWA-covered species was 1,653,345 in 1973, 1,027,450 in 2007, and 780,080 in 2018.

INTERPRETATION OF TRENDS IN USE OF DOGS FOR RESEARCH

The highest numbers of dogs (>200,000 per year) reported in the USDA statistics in the 1970s likely mark the maximum usage for such purposes in U.S. history.[3,4] The decrease in dog use from the 1980s to 2000 coincides with the rapid rise of cellular and molecular disciplines, resulting in an unprecedented paradigm shift away from animal and tissue bioassays in the biological sciences (Kinter and DeGeorge, 2016). USDA data indicate that the decrease in dog use was not offset by a commensurate increase in use of NHPs, pigs, or other AWA covered species.

[3] L. B. Kinter, personal communication, November 6, 2019. This is a surmise based on extensive personal experience in the field. There was no formal requirement for recording animal usage prior to this period.

[4] By comparison, this estimate is approximately 30 percent of the total number of dogs estimated killed annually in U.S. animal shelters. See https://www.aspca.org/animal-homelessness/shelter-intake-and-surrender/pet-statistics (accessed December 10, 2019).

Despite the decline in overall dog use for biomedical research since 1973, the use of both rodent and non-rodent species for regulated product testing has increased over the same period (Kinter and DeGeorge, 2016). Hence, most of the observed decrease in dog usage over the past 40 years likely reflects declines in use for basic and discovery research activities, as discussed in detail in Chapter 3, and the near elimination of the use of dogs for teaching (Simkin et al., 2017). It is noteworthy that NHP usage, while not approaching the historically high levels observed for dogs, has nonetheless demonstrated no downward trend from 1973 to 2018 and has increased steadily in recent years, reaching and even exceeding concurrent dog usage. This trend in the use of NHPs parallels an increase in the number of regulated products for which dogs and other non-primate species would be inappropriate subjects (e.g., certain humanized-protein-based and nucleic-acid-based therapeutics). Some of the decrease in dog use over the past 40 years may have been compensated for by increasing the use of pigs for research purposes. Unfortunately, publicly available USDA data did not provide pigs with a separate designation from farm animals until 2002. Nonetheless, pig use has been trending downwards since 2002 and is currently at approximately 50,000 per year, slightly lower than dog use.

Guinea pig and rabbit usage were, respectively, approximately 409,000 and 448,000 in 1973 and 207,000 and 237,000 in 2007, far exceeding dog usage while showing comparable declines. However, the committee is aware that guinea pig and rabbit models have replaced some dog models for product testing during this period, of which drug-induced long QT syndrome is one example (Hamlin, 2007).

Focusing on particular research uses for which dogs were once a dominant model, 10 of which are discussed in detail in Chapter 3, most (though not all) dog models have been replaced by smaller species, primarily rodents. For example, a literature search of the eight major cardiovascular research journals revealed that in the past 20 years, the overwhelming majority of papers reporting experimental animal models used rodents, mostly mice, which are not tracked by the USDA (Harrison, 2019).

CURRENT DISTRIBUTION OF LABORATORY DOG USAGE AMONG RESEARCH INSTITUTIONS

In an effort to better understand how dogs are currently being used in the United States, the committee reviewed the 1,149 annual reports submitted to the USDA APHIS under the AWA from all research facilities in all 50 states and territories in 2017, noting the number of dogs used by each type of reporting institution.

To provide context for the dog usage data, the committee also collected 2017 usage data for two other laboratory animals: NHPs, which are commonly used as a surrogate for humans for phylogenetic reasons; and pigs, which are often considered a large-animal alternative to dogs.

Procedure for Assessing Distribution of the U.S. Department of Agriculture–Regulated Dog Use

To perform its analysis, the committee used a publicly available website (USDA APHIS, n.d.a). This site enables the user to access all institutional USDA APHIS reports filed in each state in a given year. When this analysis was initiated (in mid-2019), the most recent year with complete institutional reports was 2017. Committee members performed a state-by-state review of animal usage in 2017 to obtain total dog, pig, and NHP usage for each type of institution. This review was done in three steps. First, the USDA summary of each report was viewed, to determine whether any dog, pig, or NHP usage had been reported. Second, if usage of any of these species was non-zero, then the individual report was examined and the name of the reporting institution was recorded,

along with its total dog, pig, and NHP usage (columns C + D + E in the report). Third, each institution was classified as either academic/hospital, company/private research organization, government agency (including the VA), or non-research.

For each state, the total dog usage obtained in the committee's analysis was checked by National Academies of Sciences, Engineering, and Medicine staff against the total provided by the USDA in its annual summary of 2017 data broken down by state (USDA APHIS, 2020). Wherever a discrepancy arose, the staff sought to correct it or determine its source. The sources of all discrepancies were determined, and totals were reconciled. In some cases, it was concluded that the discrepancies were due to small errors in USDA data calculating the total dog usage for a particular institution or state. As a result, the committee's sum of 60,190 dogs used in 2017 differs slightly from the total of 59,401 posted on the USDA website as of the writing of this report (USDA APHIS, n.d.a).

Results: Distribution of U.S. Department of Agriculture–Regulated Dog Use in 2017

Of the 60,190 dogs reported to USDA as used under the AWA in 2017, 22,933 were reported by 213 academic institutions and affiliated hospitals engaging in biomedical research and education (including veterinary research conducted for the benefit of dogs); 34,875 by 105 companies and private research organizations engaging in applied biomedical research and product development, including testing required by regulatory agencies; 832 by 11 government agencies (including VA research labs) conducting basic and applied research in support of their missions; and 1,550 by 16 other, non-research groups (see Table B-1).

The 2017 USDA data indicate that industry, defined as companies and private research organizations, is now the dominant user of dogs for biomedical research, exceeding the total usage by academia and government combined. The majority of industrial dog usage likely represents product safety testing designed and conducted by sponsors in fulfillment of regulatory requirements, as stipulated by the U.S. Food and Drug Administration (Kinter and DeGeorge, 2016), the U.S. Environmental Protection Agency (EPA, n.d.), other federal agencies, and international equivalents (Spielmann, 2002). These would include general toxicology studies required for the nonclinical

TABLE B-1 Usage of Dogs, Non-Human Primates (NHPs), and Pigs Reported to the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service in 2017

Animal	Institution Type	Reporting Institutions	Animals Used in 2017
Dog	Academic	213	22,933
	Industry	105	34,875
	Government	11	832
	Non-Research	16	1,550
	TOTAL	345	60,190
NHP	Academic	104	28,859
	Industry	46	37,906
	Government	12	5,200
	Non-Research	6	409
	TOTAL	168	72,374
Pig	Academic	192	25,447
	Industry	104	20,256
	Government	33	9,379
	Non-Research	6	70
	TOTAL	335	55,152

SOURCE: Publicly available data from USDA (USDA APHIS, n.d.a) were analyzed as described in the text.

development and product registration of pharmaceuticals and medical devices, agricultural and industrial chemicals, veterinary products, and household chemicals. The UK Home Office offered similar conclusions for experimental procedures performed in the United Kingdom using dogs in 2018 (Home Office, 2019). In the testing of pharmaceuticals for which the dog is selected as the non-rodent development species, a protocol that follows current guidelines will use approximately 150 dogs over 3 years, or 50 dogs per year per product (Kinter and DeGeorge, 2016). Hence, the entirety of 2017 USDA-reported dog usage by U.S. companies and private research organizations could be accounted for by roughly 700 active development projects.

In recent years, there has been increasing pressure on regulatory authorities and agencies to decrease the use of large animals in research (e.g., Grimm, 2019). The plateau in annual dog usage over the past 20 years is consistent with the fact that, despite this pressure, international requirements using non-rodent species (including dogs) in product development testing have increased, rather than decreased, over this period (Brock et al., 2013; Kinter and DeGeorge, 2016; van der Laan and DeGeorge, 2013).

Distribution of U.S. Department of Agriculture–Regulated Non-Human Primate and Pig Use in 2017

Drawing on the same institutional animal reports used to determine the distribution of dog usage, the committee examined the distribution of both NHP and pig usage among types of institutions in 2017 in order to ask whether a significant amount of research using dogs had shifted to one of these other species (USDA APHIS, n.d.).

In 2017, 46 U.S. industrial institutions reported using a total of 37,906 NHPs, exceeding the combined totals of 122 academic, government, and other institutions (see Table B-1). As noted above, the trend in use of NHPs—particularly given their predominant use in industry—is consistent with an increasing number of regulated products for which dogs and other non-primate species would be inappropriate subjects. In 2017, 104 U.S. industrial institutions reported using a total of 20,256 pigs, compared with 25,447 pigs reported by 192 academic institutions and 9,379 by 33 government institutions (see Table B-1).

Collectively, these numbers indicate (1) in the United States, dogs and NHPs are used primarily by industry to support product development; (2) the use of pigs lags behind that of dogs and NHPs in industrial organizations; and (3) academic and government institutions may have shifted some basic research activities away from dogs and NHPs to pigs.

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Appendix C

Biographical Sketches of Committee Members

Brigadier General Rhonda Cornum, Ph.D., M.D. (*Chair*), retired from the U.S. Army and joined TechWerks as the director of health strategy in 2012. Although TechWerks is a software development and psychological fitness training company, Dr. Cornum started her career as a biochemist for the Army, doing preclinical work on wound healing agents and blood preservation and amplification. After later earning an M.D., and while engaged in a urology residency, she continued research interests and tested liquid and lyophilized fibrin products as both hemostatic and local drug delivery vehicles in rabbits, pigs, and dogs. As a staff member and later the commander of several military medical centers, she has served as the director of clinical investigation and on multiple institutional animal care and use committees. More recently, Dr. Cornum served from 2009 to 2012 as the first director of the U.S. Army's novel Comprehensive Soldier Fitness initiative. This strategy represents the model for universal implementation of physical and psychological health promotion within the U.S. Department of Defense. She previously served as the assistant surgeon general for force projection, responsible for the policies and procedures to prepare soldiers and units for deployment, and she commanded the Landstuhl Regional Medical Center, the evacuation hub for Iraq, Afghanistan, Africa, and Europe. During this assignment she commissioned development of the Joint Patient Tracking Application and pioneered the use of the Nova Lung during critical care air transport. Dr. Cornum has written or co-authored one book, seven book chapters, and numerous scientific articles. She is a professor of military and emergency medicine at the Uniformed Services University of the Health Sciences. She is board certified in urology, is a fellow in both the American College of Surgeons and the Aerospace Medical Association, and is a member of the American Society of Nutrition. Her decorations include the Distinguished Service Medal, Legion of Merit (with two oak leaf clusters), Distinguished Flying Cross, Bronze Star, Meritorious Service Medal, Purple Heart, Air Medal, and Prisoner of War Medal. She holds a Ph.D. in biochemistry and nutrition from Cornell University and an M.D. from the Uniformed Services University of the Health Sciences in Bethesda, Maryland.

W. Ron DeHaven, D.V.M., M.B.A. (*Vice Chair*), has an extensive background in the veterinary profession, including 9 years as the chief executive officer of the American Veterinary Medicine Association (AVMA). At AVMA, he served more than 88,000 members and worked to meet the challenges of improving human and animal health. Mr. DeHaven also worked with the U.S. Department of Agriculture's (USDA's) Animal and Plant Health Inspection Services (APHIS) for nearly three decades, finishing his career there as the administrator, the agency's top position. At the USDA APHIS he focused on protecting U.S. agriculture and natural resources from exotic pests and diseases, handling wildlife management activities, and overseeing the Animal Welfare Act. Mr. DeHaven's work with the USDA APHIS, where he also served as the chief veterinary officer for animal health for the United States, gained him national prominence in 2003 and 2004 when bovine spongiform encephalopathy (mad cow disease) and H5N1 avian influenza were making national headlines. His public service earned him many awards, including two Presidential Rank Awards for his leadership in government, two USDA Secretary's Honor Awards, and AVMA's Meritorious Service Award. He received his D.V.M. in 1975 from Purdue University and was awarded an honorary doctor of science degree from Purdue University in 2005. After leaving AVMA, Mr. DeHaven created a veterinary consulting business with clients in academia, government, and the private sector.

Donna K. Arnett, Ph.D., M.S.P.H., B.S.N., is the dean of the University of Kentucky (UK) College of Public Health and the Norton Healthcare professor in the Department of Epidemiology. Prior to her appointment at UK in 2016, Dr. Arnett was the associate dean and the chair of the Department of Epidemiology at the University of Alabama at Birmingham. With 25 years of continuous funding from the National Institutes of Health (NIH), Dr. Arnett studies the genetic architecture of cardiovascular disease and its risk factors. A past president of the American Heart Association (AHA), she has led AHA's research committee and scientific publishing committee. Dr. Arnett is also an elected fellow of AHA, the American College of Epidemiology, and the American Epidemiological Society. She has more than 25 years of experience leading the recruitment and scientific oversight of large, multi-site cardiovascular cohort studies, including the NIH-sponsored clinical studies "The Hypertension Genetic Epidemiology Network (HyperGEN)" and "The Genetics of Lipid Lowering and Diet Network." Dr. Arnett has published more than 650 peer-reviewed papers and 2 books. In 2017 she received the Population Research Prize from AHA. She holds a B.S. in nursing and an M.S.P.H. in biostatistics and epidemiology from the University of South Florida and a Ph.D. in epidemiology from the University of North Carolina at Chapel Hill.

Warren Casey, Ph.D., DABT, is the acting chief of the U.S. National Toxicology Program's (NTP's) Biomolecular Screening Branch at the National Institute of Environmental Health Sciences (NIEHS) and the executive director of the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods, serving previously as the director of NTP's Interagency Center for the Evaluation of Alternative Toxicological Methods. These groups work together to facilitate the development, validation, regulatory acceptance, and industry adoption of non-animal test methods. Prior to joining NIEHS Dr. Casey worked at GlaxoSmithKline for 15 years in a variety of roles, including manager of pharmaceutical microbiology, head of in vitro biomarker development, and manager of discovery and investigative toxicology. Dr. Casey received his undergraduate degree in biochemistry and his Ph.D. in microbiology from North Carolina State University, where he also holds an adjunct professorship and has been named a distinguished alumnus in both the Department of Microbiology and the College of Agriculture and Life Sciences. He has been a diplomate of the American Board of Toxicology since 2007, co-chairs the OECD Validation Management Group–Non-Animal (2015–present), received the 2016 Society of Toxicology (SOT) Animal Welfare Award, and is past president of the SOT In Vitro and Alternative Methods specialty section. Dr. Casey served as a captain in the U.S. Army and Army National Guard during the Gulf War

and was awarded the National Defense Service Medal, the Southwest Asia Service Medal, and the Kuwait Liberation Medal.

Chris Green, J.D., is the executive director of Harvard Law School's Animal Law & Policy Program. He is a graduate of the Harvard Law School and the University of Illinois, where he created the school's first environmental science degree. Mr. Green is a former chair of the American Bar Association's TIPS Animal Law Committee and previously was the director of legislative affairs for the Animal Legal Defense Fund. Mr. Green currently serves on the executive board of the National Sheriffs' Association's Coalition on Violence Against Animals, previously served on the board of the National Center for Animal Law, was an advisor to the National Canine Research Council, and is a member of both the American Veterinary Medical Law Association and the Illinois Farm Bureau. Mr. Green was a member of the California Veterinary Medical Association's Non-Economic Recovery Task Force, helping explore legislative options to balance the profession's increasing liability exposure with a more equitable assessment of companion animal value. He later acted as an advisor to members of the American Veterinary Medical Association's Task Force on the Legal Status of Animals, addressing those same legislative issues at a national level. Mr. Green served on the National Academies committee that organized the Workshop on Future Directions for Laboratory Animal Law in the United States (2017–2018) and co-hosted the event at the Harvard Law School. Mr. Green has consulted on animal legal issues for CNN, CBS News, NBC News, Headline News, Politico, *The Atlantic*, *Bloomberg News*, *Harper's*, *Huffington Post*, *Science* magazine, *Smart Money* magazine, *The New York Times*, *Chicago Tribune*, *San Francisco Chronicle*, *The Wall Street Journal*, and *The Washington Post*. He also has spent the past 20 years managing an Illinois farm that has remained in his family for 180 consecutive years.

Joan C. Hendricks, V.M.D., Ph.D., served on the faculty of the University of Pennsylvania School of Veterinary Medicine for more than 20 years. Her research focused on sleep biology. She conducted National Institutes of Health (NIH)-supported biomedical studies in animals from cats to English bulldogs to fruitflies, from 1980 until becoming dean. In 2001 she was named the Henry and Corinne R. Bower Professor of Small Animal Medicine, the first woman to be named to an endowed professorship at the school. Dr. Hendricks also served as the chief of critical care in the Department of Clinical Studies at Philadelphia, she was the founding director of the Veterinary Clinical Investigation Center at the school, and she held a secondary appointment as professor in the Department of Medicine at Penn Medicine. She is a recognized expert in the field of sleep and sleep disorders and has, for decades, studied the physiology and anatomy of sleep and an animal model of sleep apnea (the English bulldog). She later switched to using *Drosophila* as a model to study sleep and sleep disorders. From 2006 to 2018 Dr. Hendricks was the Gilbert S. Kahn Dean of Veterinary Medicine at the School of Veterinary Medicine. In this capacity, she oversaw all research conducted in the school and in collaborations across Penn. Penn Vet has the largest portfolio of individual NIH-supported grants of any veterinary school in the United States. Dr. Hendricks has a B.S. in biology and psychology from Yale University and a V.M.D. and a Ph.D. from the University of Pennsylvania. Dr. Hendricks retired in August 2019. As the daughter of a career Army officer, Dr. Hendricks has a personal interest in the health and well-being of veterans.

Jonathan Kimmelman, Ph.D., is the James McGill Professor and the director of the Biomedical Ethics Unit/Social Studies of Medicine at McGill University. He has cross appointments in experimental medicine, epidemiology, biostatistics and occupational health, and human genetics. Dr. Kimmelman holds a Ph.D. in molecular biophysics and biochemistry from Yale University, and he joined McGill in 2005. His research group, Studies of Translation, Ethics, and Medicine (STREAM), uses empirical and conceptual research methods to study the ethical, social, and

policy dimensions of translational research. He received the Institute of Genetics Maud Menten New Investigator Prize, a Canadian Institutes of Health Research (CIHR) New Investigator Award (2008), and a Friedrich Bessel-Humboldt Award (2014). Dr. Kimmelman chaired the ethics committee of the American Society of Gene and Cell Therapy (2008–2010) and of the International Society of Stem Cell Research (2014–2016). He also served on the CIHR Stem Cell Oversight Committee, currently serves on several data and safety monitoring boards of the U.S. National Institutes of Health, and has been a member of three National Academies committees. His book, *Gene Transfer and the Ethics of First-in-Human Trials: Lost in Translation*, was published by the Cambridge University Press. In 2018 he was elected as a Hastings Center Fellow. Current projects being pursued in STREAM include the empirical study of prediction in clinical research and the implications of clinical trial portfolios for drug development, human protections, and clinical decision making.

Lewis B. Kinter, Ph.D., DABT, Fellow A.T.S., is currently the president and the principal scientist at GLP Scientific Consulting in Unionville, Pennsylvania. He has been engaged in pharmaceutical, biological, and medical device research and development for more than 35 years and is an internationally recognized expert in cardiovascular/renal physiology, pharmacology, toxicology, and nonclinical research and development (R&D). Dr. Kinter received his B.S. in biology at Union College (1973) and his doctorate in medical physiology from Harvard University (1978). From 1981 to 2014 he held positions of increasing responsibility in biomedical R&D with Smith Kline & French, SmithKline Beecham, Sterling Winthrop, Nycomed Amersham, Astra Merck, and AstraZeneca. Dr. Kinter is a diplomate of the American Board of Toxicology, a fellow of the Academy of Toxicological Sciences, a professor of pharmacology and toxicology (adjunct) at Michigan State University, and a former professor of physiology (adjunct) at the University of Pennsylvania School of Medicine. He has authored more than 100 research manuscripts and book chapters and organized and participated in numerous courses, workshops, symposia, and professional meetings in basic and applied physiology, pharmacology, toxicology, and nonclinical pharmaceutical R&D. Dr. Kinter currently holds memberships in the American Physiological Society (38 years), the American Society for Pharmacology and Experimental Therapeutics (31 years), the Society of Toxicology (21 years), and the Safety Pharmacology Society (18 years). He is a founder and the former president of the International Consortium for Innovation and Quality in Pharmaceutical Development, a former chairman of the Pharmaceutical Research and Manufacturers' Association Preclinical Sciences Leadership Group, and the founder and the former president of the Safety Pharmacology Society. Throughout his career Dr. Kinter has served as research scientist, co-investigator, principal investigator, study director, and department manager or director for authorized in vivo physiological, pharmacological, and toxicological investigations in vertebrates including fish, mice, rats, rabbits, ferrets, swine, dogs, and non-human primates (NHPs). He is accomplished in small animal survival surgery and championed the early use of chronically implanted vascular catheters, sensors, and telemetry devices in mice, rats, rabbits, dogs, and NHPs to improve scientific data quality and laboratory efficiency, and to reduce animal use (the Reduction and Refinement objectives in the 3 Rs). His efforts have been recognized to reduce annual animal use in susceptible applications by 75 percent or more. Dr. Kinter has been an active participant, a member, and a chair of institutional animal care and use committees in several organizations in which he was employed or volunteered. Dr. Kinter continues to serve in leadership capacities on several boards of professional scientific and charitable organizations.

Sarah L. Lathrop, D.V.M., Ph.D., is a professor of pathology at the University of New Mexico (UNM) School of Medicine, currently conducting research on infectious diseases and injury. After receiving her B.S. in animal science from Colorado State University, she earned her D.V.M. from

the University of Minnesota College of Veterinary Medicine. She practiced both small and large animal clinical veterinary medicine in Cortez, Colorado, and then completed a Ph.D. in veterinary preventive medicine at The Ohio State University, focusing on infectious disease research in cattle while also providing veterinary care for gnotobiotic research animals at the Ohio Agricultural Research and Development Center. Dr. Lathrop served a 2-year postdoctoral fellowship at the Centers for Disease Control and Prevention (CDC) as an epidemic intelligence service officer, studying vector-borne infectious diseases such as plague, tularemia, and dengue and the impact of zoonotic diseases. She then conducted vaccine research in cattle, swine, dogs, and cats at Merial's Athens Clinical Unit in Georgia, focusing on vaccine development and licensure. Dr. Lathrop joined UNM in 2003 to conduct research on infectious diseases and injury, with continuous extramural funding from CDC and, more recently, the U.S. Department of Defense. She also serves as the principal investigator for the Foodborne Diseases Active Surveillance Network portion of New Mexico's Emerging Infections Program, managing a staff of 10 researchers. She has served on numerous institutional animal care and use committees, biosafety committees, and scientific review committees for tissue repositories, as well as a reviewer for numerous peer-reviewed journals.

Nancy Figler Marks, D.V.M., M.S., DACLAM, is a diplomate of the American College of Laboratory Animal Medicine (ACLAM). She serves as the veterinarian and director of the institutional animal care and use committee (IACUC) office at The University of Iowa in Iowa City, Iowa, where she oversees the IACUC office staff and IACUC functions. She serves as a veterinary reviewer for animal protocol submissions and is a voting member of the IACUC. Dr. Marks led the development of an electronic animal protocol form to improve the review and oversight of animal research proposals. Prior to joining The University of Iowa in 2012, she held various positions of increasing responsibility with Parke Davis Pharmaceutical and Pfizer from 1998 to 2012. She received her D.V.M. from the Virginia–Maryland Regional College of Veterinary Medicine where she specialized in government and corporate medicine. Dr. Marks did her postdoctoral training at Parke Davis Pharmaceutical in conjunction with the University of Michigan in Ann Arbor, Michigan. Her areas of expertise include veterinary care of a wide variety of laboratory animal species, extensive knowledge of animal welfare regulations, inspection of animal facilities, and experience evaluating common research procedures. Additionally, Dr. Marks has an M.S. in biology from Texas A&M University. Her primary professional interest is to support compliant research involving animal models while assuring the best possible care and welfare of the animals. She has developed content and lectured in the American Association for Laboratory Animal Science, ACLAM, and the Public Responsibility in Medicine and Research symposia, conferences, courses, and workshops relevant to the role of the IACUC, animal protocol development and review, decreasing regulatory burden while maintaining animal welfare, methods for promoting animal welfare, implementing the 3 Rs, as well as aspects of human safety in the vivarium. Dr. Marks has also authored and co-authored papers related to sea turtle physiology and behavior, protein biochemistry, and various laboratory animal topics.

Christian E. Newcomer, V.M.D., M.S., DACLAM, is a 1977 graduate of the School of Veterinary Medicine at the University of Pennsylvania. Following 1 year as a research associate/large animal intern at The Pennsylvania State University (1977–1978), he entered postdoctoral training in laboratory animal medicine at the University of Michigan (1978–1981) and became board certified as a diplomate in the American College of Laboratory Animal Medicine (ACLAM) in 1982. During his career he held clinical, academic, and leadership positions in laboratory animal medicine at the Massachusetts Institute of Technology (1981–1987), Tufts–New England Medical Center and the Tufts University School of Veterinary Medicine (1987–1994), the University of North Carolina at Chapel Hill (1994–2001), the Veterinary Resources Program at the National

Institutes of Health (2001–2003), and Johns Hopkins University (2003–2008). In 2008 he joined AAALAC International as its executive director, capping more than 25 years of involvement with that organization as an ad hoc site visitor and member of the Council on Accreditation; he retained the title of executive director emeritus upon retirement in 2016. He is a past-president of ACLAM (1996) and of the American Association for Laboratory Animal Science (2008) and the vice president of the AAALAC International Council on Accreditation (1996–1998). He served as a member of the Institute for Laboratory Animal Research's Committee on Occupational Health and Safety in Research Animal Facilities (1993–1996) and the chairman of the Committee on Cost of and Payment for Animal Research Institute for Laboratory Animal Research (1997–2000). He also has authored 26 peer-reviewed articles and 22 book chapters and has spoken extensively on many topics to promote the quality of care for research animal subjects and the discussion of the ethical considerations of their use.

William Z. Potter, M.D., Ph.D., earned his B.A., M.S., M.D., and Ph.D. at Indiana University, after which he functioned in positions of increasing responsibility and seniority over the next 25 years at the National Institutes of Health (NIH) with a research focus on translational neuroscience. While at NIH, Dr. Potter was widely published and appointed to many societies, committees, and boards; these roles enabled him to develop a wide reputation as an expert in psychopharmacological sciences and for championing the development of novel treatments for central nervous system (CNS) disorders. Dr. Potter left NIH in 1996 to accept a position as the executive director for early clinical neuroscience at Lilly Research Labs, and in 2004 he joined Merck Research Labs (MRL) as the vice president of clinical neuroscience, after which he moved to the newly created position of translational neuroscience in 2006, a position from which he retired in January 2011. His experience at Lilly and MRL in identifying, expanding, and developing methods of evaluating CNS effects of compounds in human brain cover state-of-the-art approaches across multiple modalities. These include brain imaging and cerebrospinal fluid proteomics as well as development of more sensitive clinical measures. Dr. Potter continues as an emeritus co-chair of the Neuroscience Steering Committee of the Foundation for NIH and served through 2019 as a senior advisor to the director of the National Institute of Mental Health, where he has championed the position that more disciplined hypothesis testing of targets in humans through public–private partnerships is the best near-term approach to moving CNS drug development forward for important neurologic and psychiatric illnesses.

David M. Powell, Ph.D., joined the Saint Louis Zoo as the director of research in August 2016. He is responsible for the oversight of behavioral, reproductive, and endocrine research as well as some visitor studies research. Prior to coming to Saint Louis, Dr. Powell was the associate curator of mammals at the Wildlife Conservation Society's Bronx Zoo in New York for 12 years, where he developed a strong background in captive mammal management and husbandry. Dr. Powell received his Ph.D. from the University of Maryland for his studies of behavior and reproductive biology in the feral horses on Assateague Island. Dr. Powell did his postdoctoral studies at the Smithsonian Institution's National Zoological Park in the Department of Conservation Biology studying giant panda behavior in U.S. and Chinese zoos for 4 years. Dr. Powell also worked at Zoo Atlanta from 1988 to 1993 in various roles, including animal keeper, animal diet technician, and research intern. He is actively involved in the Association of Zoos & Aquariums (AZA) professional activities, including serving on Taxon Advisory Group steering committees, managing breeding programs, and serving on the AZA Animal Welfare and Wildlife Conservation & Management Committee. He currently serves on the Research Review Committee of the Saint Louis Zoo. Previously, he served on the institutional animal care and use committees of Lehman College (2008–2014) and the National Zoo, Rock Creek Campus (2004). Dr. Powell's research has focused on a number of species and

topics over the years in zoos and in the field. Topics of study have included dominance in animal societies, reproductive competition, maternal behavior, impacts of environmental enrichment and other husbandry practices on behavior, animal welfare, the characterization of animal personality and personality measurement methods, the affective impact of zoo exhibits on visitors, and studies of animal care staff attitudes about population management practices.

Margaret (Mimi) Foster Riley, J.D., is a professor at the University of Virginia (UVA) School of Law, with a secondary appointment at the School of Medicine and a program affiliation with the Batten School of Leadership and Public Policy. She teaches food and drug law, health law, animal law, bioethics, the regulation of clinical research, and public health law. Ms. Riley has written and presented extensively about health care law, biomedical research, genetics, reproductive technologies, stem cell research, animal biotechnology, health disparities, and chronic disease. She is the director of UVA's Program in Animal Law. She serves as the chair of UVA's Embryonic Stem Cell Research Oversight Committee and as the legal advisor to the Health Sciences Institutional Review Board. She served on the National Research Council Committee on Revisions to the Common Rule for the Protection of Human Subjects and the National Academies Committee on Controlled Human Exposure Studies at the U.S. Environmental Protection Agency and was a consultant on the National Academies Committee on Pain Management and Regulatory Strategies to Address Prescription Opioid Abuse. She has advised numerous committees of the National Academy of Medicine and the Virginia Bar. Before coming to Virginia, Ms. Riley was an associate with Pepper Hamilton & Scheetz in Philadelphia, where she worked primarily in complex securities, commercial and mass tort litigation. Prior to that position, she was a litigation associate with Rogers & Wells in New York. Ms. Riley received her law degree from Columbia University and her bachelor of arts from Duke University.

Rodney A. White, M.D., is the director of vascular surgery services at the Long Beach MemorialCare Heart & Vascular Institute in Long Beach, California and, prior to that, was the chief of vascular surgery at the Harbor–University of California, Los Angeles (UCLA), Medical Center in Torrance, California. His academic appointment is emeritus professor of surgery at the UCLA School of Medicine. He is certified by the American Board of Surgery with special qualifications in general vascular surgery and by the American Board of Laser Surgery. He also has a permit as a fluoroscopy supervisor and operator from the State of California and is a registered vascular technologist certified by the American Registry of Diagnostic Medical Sonographers. Dr. White's research interests include the development and evaluation of artificial implant materials and laboratory and clinical investigation of fundamental problems and new procedures in vascular surgery. He is co-inventor of a process for fabricating microporous biomaterials, including an artificial bone substitute that was recently awarded the first annual U.S. Congressional Golden Goose Award for federally funded research that has led to significant patient care and economic benefit. Current research programs involve the development and evaluation of endovascular surgical devices including atherectomy devices, stents, abdominal and thoracic endoluminal prostheses, and angioscopy and intraluminal ultrasound imaging technologies. Dr. White is the recipient of research grants from the National Institutes of Health, the American Heart Association, and numerous national clinical studies. Dr. White is the author of more than 300 papers and 200 book chapters and is the co-author or editor of 12 books addressing a broad spectrum of topics in vascular and endovascular surgery. He is the co-editor of the *Journal of Endovascular Therapy*. He is a member of several industrial and governmental panels evaluating new medical technologies. He is the past vice-chairman of the board of directors of the Los Angeles Biomedical Research Institute at Harbor–UCLA Medical Center. Dr. White is an active member of 15 regional, national, and international societies and is the past-president of the International Society for Endovascular Specialists and the past secretary of the Society for Vascular Surgery.