REPLACEMENT OR REDUCTION OF GENE-EDITED ANIMALS IN BIOMEDICAL RESEARCH: A COMPARATIVE ETHICS AND POLICY ANALYSIS*

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Since William Bateson's 1906 coinage of the term "genetics," the rise of mice as a model organism for biomedical research has gone hand in hand with genomic developments. In today's research environment, mice and rats make up the vast majority of all research subjects. While the advent of gene-editing tools such as CRISPR has made genetic manipulation of mice easier, these tools also signal a new trend toward an increased use of large-animal models such as dogs, pigs, and nonhuman primates. Especially for neurological impairments, CRISPR gene editing offers the potential to generate large-animal models that better mimic human diseases. What are the ethical and regulatory implications of this trend? The professional and ethical framework for responsible conduct of animal research is widely recognized as the "three Rs": Reduction, Refinement, and Replacement. This Article points to the tension between reduction (decreasing the overall numbers of animals used) and relative replacement (the use of mice and rats instead of species with more "complex" capacities) that is implied by such a trade-off. The Article argues, however, that a comparative analysis of regulatory frameworks in the United States and in the European Union shows that neither offers any substantial guidance to moderate a trend toward greater use of large-animal models.

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Nevertheless, we raise several ethical questions associated with the trend toward using relatively fewer animals but replacing less cognitively developed animals with those with potentially greater morally relevant capacities and moral standing.

INTRODUCTION

In today's biomedical research environment, mice and rats account for the vast majority of animals used.[1] While the advent of new gene-editing tools such as Clustered Regularly Interspaced Short

1. *See* EUROPEAN COMM'N, REPORT FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT: SEVENTH REPORT ON THE STATISTICS ON THE NUMBER OF ANIMALS USED FOR EXPERIMENTAL AND OTHER SCIENTIFIC PURPOSES IN THE MEMBER STATES OF THE EUROPEAN UNION 3 (2013), https://eur-lex.europa.eu/resource.html?uri=cellar:e99d2a56-32fc-4f60-ad69-61ead7e377e8.0001.03/DOC_1&format=PDF [https://perma.cc/72B2-JF85]. Because only some countries collect statistics on the numbers of animals used generally, and rodents in particular, estimates of actual percentages of mice and rats vary as compared to other mammals. Katy Taylor et al., *Estimates for Worldwide Laboratory Animal Use in 2005*, 36 HUMANE SOC'Y INST. FOR SCI. & POL'Y ANIMAL STUD. REPOSITORY 327, 327–28 (2008).

Palindromic Repeats ("CRISPR") paired with specific nucleases (e.g., Cas) has made genetic manipulation of mice easier, they also portend a new trend toward an increased use of large-animal models such as dogs, pigs, and nonhuman primates ("NHPs"), among others.[2] These gene-editing tools offer the potential to generate large-animal models that better mimic human diseases and are thus potentially more translatable to human medicine.[3]

The responsible conduct of animal research has largely been based on the idea of the "three Rs" of reduction, replacement, and refinement of animal research.[4] Reduction aims to minimize the number of animals used to those needed for the scientific endeavor. Replacement works to substitute the use of live animals with alternative models, such as computer-based simulation or in vitro studies. Refinement involves lessening harms to animals used in research through changes to research procedures, improved management of pain and distress, and improved housing and caretaking. Within the broader replacement framework, partial or relative replacement envisions substituting cognitively higher-developed animals with cognitively less-developed animals.[5] This is based on the idea that more cognitively developed species have an increased sentience and a greater ability to suffer.[6]

A core professional ethics problem regarding the greater use of gene-edited large-animal models is the tension between reduction and relative replacement. According to relative replacement, there would typically be an incentive to replace larger animals with smaller species if they are also less cognitively developed.[7] However, since far fewer

2. *See* Aaron C. Ericsson, Marcus J. Crim & Craig L. Franklin, *A Brief History of Animal Modeling*, 110 MO. MED. 201, 203–04 (2013). The advances of gene editing are especially remarkable given that the study of genetics is itself a relatively new academic specialty, as William Bateson coined the term "genetics" only in 1906. KAREN RADER, MAKING MICE: STANDARDIZING ANIMALS FOR BIOMEDICAL RESEARCH, 1900–1955, at 27–28 (2004).

3. Alexandra Wendler & Martin Wehling, *The Translatability of Animal Models for Clinical Development: Biomarkers and Disease Models*, 10 CURRENT OPINION PHARMACOLOGY 601, 605 (2010).

4. *See generally* W. M. S. RUSSELL & R. L. BURCH, THE PRINCIPLES OF HUMANE EXPERIMENTAL TECHNIQUE 64 (1959) (discussing "the ways in which inhumanity can be . . . diminished or removed . . . under the three broad headings of Replacement, Reduction, and Refinement" (emphasis omitted)).

5. Nicole Fenwick, Gilly Griffin & Clément Gauthier, *The Welfare of Animals Used in Science: How the "Three Rs" Ethic Guides Improvements*, 50 CANADIAN VETERINARY J. 523, 523 (2009).

6. *See id.*

7. *Id.*

large animals would be used in gene-edited disease-model studies than, for example, the number of mice that would be used in these studies, reduction argues in favor of using gene-edited large-animal models.[8] How should this tension between reduction and relative replacement be resolved? To put the point in sharp relief: If possible, should we trade a study using one thousand rodents for a potentially more translatable study using ten NHPs, dogs, or pigs?

The three Rs have been crucial to structuring regulatory guidance for animal research, especially in the European Union but also in the United States.[9] In this Article, we investigate the comparative regulatory implications in the United States and European Union of genetically modifying large animals to better model human diseases and the increased research uses of large animals that might follow. Outside of specific restrictions regarding the use of some species (particularly great apes and, to a much lesser extent, other NHPs), there is little within the U.S. or E.U. regulatory and professional ethics frameworks that offers guidance in terms of the ethical permissibility of the trade-offs between using large numbers of rodents and small numbers of large-animal models that we suggest may be on the horizon. While there is no substantive regulatory guidance on this issue, there is reason to believe that the general ethos of animal research ethics would favor the trend. At the same time, other considerations, such as the cost of research conducted with large animals and difficulties in procuring species of NHP, may dampen this trend somewhat.[10] Since regulatory guidance

8. *Id.*

9. Council Directive 2010/63, pmbl. ¶ 11, 2010 O.J. (L 276) 33, 34 [hereinafter Council Directive 2010/63]; Larry Carbone, *Pain in Laboratory Animals: The Ethical and Regulatory Imperatives*, 6 PLOS ONE, no. e21578, Sept. 7, 2011, at 1, 1–2.

10. Emily W. Lankau et al., *Use of Nonhuman Primates in Research in North America*, 53 J. AM. ASS'N FOR LABORATORY ANIMAL SCI. 278, 280–81 (2014). It is too early to tell how prevalent the trend toward greater use of large-animal models will become. According to data available through the U.S. Department of Agriculture on numbers of animals used in research, a comparison between 2012 (when CRISPR-Cas9 was developed specifically as a gene-editing tool) and 2017 (the most recent year for which data is available) shows the numbers of dogs used rose by less than 1%, sheep use rose by approximately 6%, and pig use dropped by approximately 11%. However, NHP use rose by approximately 15%. *Compare* ANIMAL & PLANT HEALTH INSPECTION SERV., USDA, ANNUAL REPORT ANIMAL USAGE BY FISCAL YEAR 2012 (2014) [hereinafter ANNUAL REPORT ANIMAL USAGE BY FISCAL YEAR 2012], www.aphis.usda.gov/animal_welfare/downloads/reports/Animals%20Used%20In%20Research%202012.pdf [https://perma.cc/BH5F-5X8L], *with* ANIMAL & PLANT HEALTH INSPECTION SERV., USDA, ANNUAL REPORT ANIMAL USAGE BY FISCAL YEAR 2017 (2018) [hereinafter ANNUAL REPORT ANIMAL USAGE BY FISCAL YEAR 2017], https://www.aphis.usda.gov/animal_welfare/

on an increased use of genetically modified large mammals within biomedicine is sparse, we do briefly note broader social and ethical conundrums that the trend raises.

The discussion proceeds in five parts. Part I introduces the scientific landscape in which large-animal models play an increasingly important role in research. Part II focuses on the regulatory and professional ethical frameworks guiding animal research in the United States and in the European Union, particularly emphasizing differences between the two jurisdictions that suggest inconsistencies in their moral approach to animal research. Part III introduces the issue of moral standing for the animals subject to genetic and other biomedical studies and illustrates how the U.S. and E.U. regulatory frameworks approach the issue. Part IV then analyzes the implications of these policy differences for the use of gene-edited large-animal models. Finally, Part V introduces moral considerations that have not yet been accommodated in the regulatory and professional ethical frameworks governing gene-edited animal research to offer some introductory thoughts on how existing frameworks could be improved.

I. GENE EDITING AND EFFICIENT AND EFFECTIVE LARGE-ANIMAL MODELS

The scientific basis for a potential turn to a greater use of gene-edited large-animal models consists of two factors. First, there is broad agreement that there is a need to improve current animal models to increase efficiency and translatability of animal research.[11] While rodents have long served as models for human diseases, knowledge gained through their use in biomedical research may be less translatable to humans than knowledge gained through the use of larger mammals that are otherwise generally more similar to humans.[12] Second, CRISPR gene editing represents a new tool with unprecedented capacity to efficiently and effectively manipulate the

downloads/reports/Annual-Report-Animal-Usage-by-FY2017.pdf [https://perma.cc/S9CC-D3C9]. Importantly, it is impossible to tell what portion of that use is related to genome editing. *Compare* ANNUAL REPORT ANIMAL USAGE BY FISCAL YEAR 2012, *supra*, *with* ANNUAL REPORT ANIMAL USAGE BY FISCAL YEAR 2017, *supra*.

11. *See* Hideyuki Okano & Noriyuki Kishi, *Investigation of Brain Science and Neurological/Psychiatric Disorders Using Genetically Modified Non-Human Primates*, 50 CURRENT OPINION NEUROBIOLOGY 1, 1 (2018).

12. *Id.*

genomes of animal models, including large mammals.[13] Because larger animals have significant potential to better model human diseases than smaller animals and are typically used in much fewer numbers than smaller animals, they offer the potential for a dramatic change in the translatability and efficiency of animal research.[14]

Rodents are easy to breed, relatively short-lived, efficient to house and manage, and there are well-established gene-manipulation techniques for these animals.[15] The expanded use of mice in the early decades of genetics also largely managed to escape social critique in part because the initial uses were primarily for genetic breeding purposes and also because the antivivisectionist movement of the time was focused on scientists' use of dogs and cats.[16] These factors all contribute to mice being a model of choice for many biomedical researchers today.

Limitations in the model are nevertheless becoming more widely recognized and discussed. For example, while rodents have contributed greatly to our understanding of human biology and development, there are also significant differences between mice and humans that limit their usefulness as models of human diseases.[17] These differences include physical size, life span, diet, differences in brain and other organ structure and function, and genomic differences. Each of these factors may limit the translational potential of rodent studies to human clinical application.[18] Mouse studies of diseases such as cystic fibrosis, Lesch-Nylan syndrome, and Huntington's disease illustrate the shortcomings of the mouse model, as animals carrying genetic mutations relevant to the human disease phenotype nevertheless do not show all the same symptoms that a

13. Ellen Shrock & Marc Güell, *CRISPR in Animals and Animal Models*, *in* 152 PROGRESS IN MOLECULAR BIOLOGY AND TRANSLATIONAL SCIENCE 95, 95–114 (Raúl Torres-Ruiz & Sandra Rodriguez-Perales eds., 2017) (discussing large mammal applications and noting CRISPR has "revolutionized the field of genome editing").

14. *Id.*

15. *See* Herbert C. Morse III, *Building a Better Mouse: One Hundred Years of Genetics and Biology*, *in* 1 THE MOUSE IN BIOMEDICAL RESEARCH: HISTORY, WILD MICE, AND GENETICS 1, 7–8 (James G. Fox et al. eds., 2d ed. 2007).

16. RADER, *supra* note 2, at 35–37.

17. Robert L. Perlman, *Mouse Models of Human Disease: An Evolutionary Perspective*, EVOLUTION, MED. & PUB. HEALTH, April 12, 2016, at 170, 170.

18. *Id.* at 171–74; *see also* Okano & Kishi, *supra* note 11, at 1; H. Bart van der Worp et al., *Can Animal Models of Disease Reliably Inform Human Studies?*, 7 PLOS MED., no. e1000245, Mar. 30, 2010, at 1, 5–6.

human will show.[19] Thus, what we learn with regard to treating the symptoms in the animal model may be of limited help for the human clinic.

Critically, a significant percentage of potential new drugs are removed from development because of a lack of efficacy and/or safety when tested in humans.[20] High attrition rates have been reported for multiple fields, including neurological diseases, oncology, and infectious diseases.[21] While there are diverse explanations for these high rates of drug attrition, including poor study design, inadequate animal models of the human diseases are frequently cited.[22]

The low rates of translatability explain why there is an increasing interest in using larger-animal models that may better mimic the course and potential cures of human diseases.[23] While multiple large-animal models have been used in the study of diseases with genomic factors, dogs, pigs, and NHPs have all been singled out as particularly promising for certain types of diseases.[24] Generally speaking, the life span of larger mammals is more similar to humans, a factor that is important for the onset and development of many human disease

19. C. Bruce A. Whitelaw et al., *Engineering Large Animal Models of Human Disease*, 238 J. PATHOLOGY 247, 247 (2015).

20. Paul McGonigle & Bruge Ruggeri, *Animal Models of Human Disease: Challenges in Enabling Translation*, 87 BIOCHEMICAL PHARMACOLOGY 162, 163 (2014); van der Worp et al., *supra* note 18, at 1.

21. Jarrod Bailey, Michelle Thew & Michael Balls, *An Analysis of the Use of Animal Models in Predicting Human Toxicology and Drug Safety*, 42 ALTERNATIVES TO LABORATORY ANIMALS 181, 196 (2014); Ismail Kola & John Landis, *Can the Pharmaceutical Industry Reduce Attrition Rates?*, 3 NATURE REVIEWS DRUG DISCOVERY 711, 712–14 (2004); Ian Walker & Herbie Newell, *Do Molecularly Targeted Agents in Oncology Have Reduced Attrition Rates?*, 8 NATURE REVIEWS DRUG DISCOVERY 15, 16 (2009).

22. *See* Melanie L. Graham & Mark J. Prescott, *The Multifactorial Role of the 3Rs in Shifting the Harm-Benefit Analysis in Animal Models of Disease*, 759 EUR. J. PHARMACOLOGY 19, 21, 23–24 (2015); *see also* Jack W. Scannell et al., *Diagnosing the Decline in Pharmaceutical R&D Efficiency*, 11 NATURE REVIEWS DRUG DISCOVERY 191, 194–95 (2012).

23. *See* Ericsson et al., *supra* note 2, at 203.

24. Margaret Casal & Mark Haskins, *Large Animal Models and Gene Therapy*, 14 EUR. J. HUM. GENETICS 266, 267 (2006); Whitelaw et al., *supra* note 19, at 247. *See generally* Sandrine Camus et al., *Why Bother Using Non-Human Primate Models of Cognitive Disorders in Translational Research?*, 124 NEUROBIOLOGY LEARNING & MEMORY 123 (2015) (explaining that NHPs particularly "remain critical for the accumulation of biomedical knowledge given that they are the closest resemblance to humans in aspects of anatomy, physiology, immunology, social behaviours, and cognitive function").

phenotypes.[25] For specific large-animal models, there are also other close similarities to humans, including organ physiology, brain structures, and behavioral and social capacities.[26] Pigs are used in diabetes research, for example, because of certain physiological similarities to humans. Current bioengineering of pigs at a molecular level to more closely mimic human diabetes is considered highly promising for biomedicine.[27] NHPs, on the other hand, may better mimic human neurodegenerative diseases due to similarities in brain structure.[28] The primate prefrontal cortex is a recently evolved brain structure responsible for higher cognitive processes and is vulnerable with regard to some psychiatric diseases.[29] Because rodent brains do not share the unique structure and function of the primate prefrontal cortex, it is impossible to fully model the complexity of the human brain in rodents.[30] Thus, even genetically modified rodents may not adequately model some human neurodegenerative diseases. For example, while a Parkin gene mutation in humans is a common cause of early-onset familial Parkinson's disease, a similar mutation of the Parkin gene in mice leads to incomplete mimicking of the Parkinson's disease symptoms in these patients.[31] Rodent models of other neurodegenerative diseases, such as Alzheimer's and Huntington's diseases, show similar limitations by lacking the typical neurodegeneration that is so significant in human patients.[32]

In sum, the turn to genome-edited large-animal models of disease is driven in part by a promise of better translation to treatment of certain human diseases and conditions. What makes the

25. *See* Ilaria Bellantuono & Paul K. Potter, *Modelling Ageing and Age-Related Disease*, 20 DRUG DISCOVERY TODAY 27, 27, 31 (2016) (noting the "complex problem" of modeling diseases in smaller animals and the insufficiency of data without models of longer life span).

26. So Gun Hong et al., *The Role of Nonhuman Primate Animal Models in the Clinical Development of Pluripotent Stem Cell Therapies*, 24 MOLECULAR THERAPY 1165, 1165 (2016).

27. Eckhard Wolf et al., *Genetically Engineered Pig Models for Diabetes Research*, 23 TRANSGENIC RES. 27, 27 (2014).

28. Camus et al., *supra* note 24, at 124–25; Casal & Haskins, *supra* note 24, at 267; Whitelaw et al., *supra* note 19, at 247.

29. Okano & Kishi, *supra* note 11, at 1.

30. *Id.*

31. *See* Jean-Michel Itier et al., *Parkin Gene Inactivation Alters Behaviour and Dopamine Neurotransmission in the Mouse*, 12 HUM. MOLECULAR GENETICS 2277, 2285–86 (2003).

32. Zhuchi Tu et al., *CRISPR/Cas9: A Powerful Genetic Engineering Tool for Establishing Large Animal Models of Neurodegenerative Diseases*, 10 MOLECULAR NEURODEGENERATION, no. 35, Aug. 4, 2015, at 1, 1–2.

turn possible, however, are the significant improvements in genome-editing technologies. Earlier methods of producing genetically modified large-animal models of human diseases offered generally low rates of success and were highly inefficient.[33] However, the newer gene editors using site-specific nucleases are able to better target loci within the genome to both "knock-in" and "knockout" specific genes.[34] In particular, CRISPR paired with specific nucleases[35] potentially offers an effective and efficient way of cheaply modifying DNA at specific locations and has led to the development of multiple new animal models with more extensive genomic modifications than earlier methods.[36] Because these newer gene editors are efficient in introducing accurate mutations in both alleles of the same gene and can be used directly in reproductive cells, they make possible the development of larger animal models of specific human diseases that were previously impossible, including in sheep, pigs, dogs, and NHPs.[37] In sum, CRISPR gene editing is seen as a potent strategy to broaden "the repertoire of useful animal disease models significantly beyond that currently available."[38]

The scientific promise of large-animal models, along with the technological capacity to generate these models, helps to explain why a turn to their increased use may be on the horizon. Yet animals used in biomedical research may undergo painful and distressing interventions, are commonly euthanized at the conclusion of a study (or at a humane endpoint to a study), and live their lives confined to a research facility.[39] Insofar as the use of animals in biomedical research

33. James West & William Warren Gill, *Genome Editing in Large Animals*, 41 J. EQUINE VETERINARY SCI. 1, 2 (2016).

34. Whitelaw et al., *supra* note 19, at 248.

35. The CRISPR/Cas9 system utilizes nuclease (protein that cuts DNA) and a guiding sequence (genetic base pairs that direct the nuclease to the gene locus of choice) that can efficiently cut DNA at targeted sites to induce mutations or to introduce specific DNA sequences into said target locus. *See* Mazhar Adli, *The CRISPR Tool Kit for Genome Editing and Beyond*, 9 NATURE COMM., no. 1911, May 15, 2018, at 1, 2–3 (outlining the development of gene-editing science).

36. Shrock & Güell, *supra* note 13, at 95–114.

37. Jon Cohen, *In Dogs, CRISPR Fixes a Muscular Dystrophy*, 361 SCIENCE 835, 835 (2018); Xiangyu Guo & Xiao-Jiang Li, *Targeted Genome Editing in Primate Embryos*, 25 CELL RES. 767, 767–68 (2015); Carolin Perleberg, Alexandre Kind & Angelika Schnieke, *Genetically Engineered Pigs as Models for Human Disease*, 11 DISEASE MODELS & MECHANISMS, no. 030783, Jan. 22, 2018, at 1, 1; Whitelaw et al., *supra* note 19, at 247; Diarra K. Williams et al., *Genetic Engineering a Large Animal Model of Human Hypophosphatasia in Sheep*, 8 SCI. REP., no. 16945, Nov. 16, 2018, at 1, 1–2.

38. Whitelaw et al., *supra* note 19, at 253.

39. *See* Council Directive 2010/63, *supra* note 9, pmbl. ¶¶ 14–15, at 34.

is justifiable, it is arguable that, at a minimum, a professional ethical responsibility exists to maximize the potential benefits from such use. From a responsible conduct-of-research point of view, then, the increase in efficiency and translatability that large-animal models seem to offer suggests a strong argument in favor of using these models. At the same time, using these animals is typically seen as morally problematic because of their relatively high social, intellectual, and emotional capacities—capacities that more closely mirror our own. In what follows, this Article analyzes the implications of a trend toward using more gene-edited large-animal models from a regulatory and professional ethics framework.

II. THE U.S. AND E.U. REGULATORY FRAMEWORKS FOR ANIMAL RESEARCH

This Article applies a comparative analysis to the animal research regulatory frameworks of the United States and European Union. These two regulatory structures are among the most stringent and influential in the world with regard to research oversight and accordingly present an opportunity to examine the extent to which helpful guidance is available on the CRISPR-driven turn to large-animal uses. As we discuss, the E.U. framework offers somewhat stronger language for animal protection and valuation and contains elements not found in the U.S. legislation, such as a required harm-benefit analysis and an upper limit on allowable pain and distress.[40] Nevertheless, as we analyze these and other elements of each framework, we reveal that neither the E.U. nor the U.S. framework offers particular guidance on the use of larger versus smaller gene-edited animal models. Accordingly, the dilemma we raise in this Article regarding the tension between reduction and relative replacement remains unaddressed. In particular, neither the United States nor the European Union would prohibit—and both might be viewed as conducive to—the turn to greater use of gene-edited larger animals for biomedical research.

A. E.U. Regulation

In the European Union, research using nonhuman animals is guided by Directive 2010/63 on the Protection of Animals Used for Scientific Purposes ("Directive 2010/63").[41] Directive 2010/63

40. *Id.* pmbl. ¶ 23, art. 38, at 35, 46–47.
41. *Id.* pmbl. ¶ 1, at 33.

harmonizes animal research legislation across E.U. member-states and was implemented in the form of national laws by each state in 2013.[42] It covers research uses of vertebrate animals and cephalopods, as well as fetal forms of mammals in the last third of their development.[43] Animal research proceeds through approval from authorities appointed in each member-state.[44] Referred to as "competent authorities," these officials make decisions about whether to authorize research projects based on a harm-benefit analysis and under advisement of an ethical review committee, among other criteria.[45] Also, a national committee of each E.U. member-state advises both the competent authority and the local animal-welfare groups and serves as a clearinghouse for information on best practices for "acquisition, breeding, accommodation, [and the] care and use of animals in procedures."[46]

B. U.S. Regulation

In the United States, the regulatory structure guiding animal research is somewhat more complex because there is both a federal law, the Animal Welfare Act ("AWA"),[47] as well as the Public Health Service ("PHS") policy, revised in 2015,[48] that covers federally funded research as mandated by the Health Research Extension Act of 1985.[49] The AWA is administered by the United States Department of Agriculture ("USDA"), specifically the Animal Care unit within the USDA's Animal and Plant Health Inspection Service ("APHIS"), and covers a wide range of issues in the transportation, sale, and handling of animals generally.[50] The AWA includes standards for humane care and use for animals in a wide variety of contexts far

42. *Id.*
43. *Id.* pmbl. ¶¶ 8–9, at 34.
44. *Id.* pmbl. ¶ 48, at 37.
45. *Id.*
46. *Id.* art. 49, at 49.
47. 7 U.S.C. §§ 2131–2159 (2012).
48. NAT'L INSTS. OF HEALTH, U.S. DEP'T OF HEALTH & HUMAN SERVS., PUBLIC HEALTH SERVICE POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS 7 (2015) [hereinafter PHS POLICY], https://olaw.nih.gov/sites/default/files/PHSPolicyLabAnimals.pdf [https://perma.cc/9G8D-BUUE].
49. *See* 42 U.S.C. § 289(d) (2012).
50. ANIMAL & PLANT HEALTH INSPECTION SERV., USDA, ANIMAL WELFARE ACT AND ANIMAL WELFARE REGULATIONS 1 (2017), https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_FINAL_2017_508comp.pdf [https://perma.cc/3JQD-DD2T].

beyond animal research.[51] By its terms, the AWA extends protections to research animals, including warm-blooded vertebrate animals, *except* birds, mice, and rats bred for research.[52] The PHS policy is administered by the National Institutes of Health's Office of Laboratory Animal Welfare ("OLAW").[53] It covers all research uses, experimentation, research training, and biological testing of live vertebrate animals sponsored (or conducted) by PHS agencies.[54] Unlike the E.U. legislation, it does not cover any fetal forms of animals nor does it cover any invertebrates.[55]

The AWA and PHS policies are similar in a number of ways, perhaps most significantly by requiring oversight of research by Institutional Animal Care and Use Committees ("IACUCs")—though they impose somewhat different membership requirements—and through the requirement for adequate veterinary care.[56] IACUCs review the research facility's programs, inspect animal facilities and laboratories, and approve individual research protocols among other duties.[57] The PHS policy also requires conformity of research practices with the *Guide for the Care and Use of Laboratory Animals* ("the Guide")[58] and with the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (the "U.S. Government Principles").[59]

An important difference between the AWA and PHS regulations pertains to facility inspection requirements. The USDA conducts unannounced on-site inspections of facilities doing research with covered species at least yearly, while the PHS policy relies on inspections of the facilities (at least every six months) by IACUCs and a written assurance submitted by the institution regarding their

51. 7 U.S.C. § 2144 (2012).
52. *Id.* § 2132(g).
53. PHS POLICY, *supra* note 48, at 9.
54. *Id.* at 7.
55. *Id.* at 8.
56. 7 U.S.C. § 2143(a) (2012).
57. 9 C.F.R. § 2.31 (2018).
58. *See generally* NAT'L RESEARCH COUNCIL OF THE NAT'L ACADS., GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS (8th ed. 2011) [hereinafter GUIDE FOR CARE AND USE], https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf [https://perma.cc/DSG2-GKHH] (addressing ethical research practices with animals in accordance with the three Rs).
59. U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, 50 Fed. Reg. 20,864, 20,864 (May 20, 1985).

compliance with PHS policies.[60] Another significant difference is which animals are covered. Because mice and rats bred for research are not covered by the AWA, their use is not reported to the USDA. Therefore, it remains unknown precisely how many of these animals are used for research in the United States. However, because estimates are that eighty-five to ninety-five percent of vertebrate animals used are mice or rats, it is significant that these animals are not covered by the AWA.[61] In the United States, therefore, certain vertebrate animals used for privately funded research in a facility not voluntarily accredited might receive no oversight protections.

C. Nongovernmental Regulation

A third aspect of the oversight picture of animal research internationally is the option of voluntary accreditation and assessment by the Association for Assessment and Accreditation of Laboratory Animal Care International ("AAALAC International"), a private nonprofit organization founded in 1965 that promotes uniform high standards of animal care and use.[62] AAALAC International accreditation requires an application process and an initial site visit.[63] Maintaining accreditation involves yearly updates as well as site visits (with notice) every three years.[64] AAALAC International assessments rely on both U.S. and E.U. guidelines, though the accreditation generally resembles the Guide in the United States by using a performance standard that looks to outcomes and professional judgment for its assessment of research programs.[65]

60. NAT'L RESEARCH COUNCIL OF THE NAT'L ACADS., SCIENCE, MEDICINE, AND ANIMALS 33–34 (2004) [hereinafter SCIENCE, MEDICINE, AND ANIMALS].

61. The U.S. National Association for Biomedical Research, a lobbying group that advocates for the use of animals in research, estimates ninety-five percent of vertebrates used are mice and rats. *Mice and Rats*, NAT'L ASS'N FOR BIOMEDICAL RES., https://www.nabr.org/biomedical-research/laboratory-animals/species-in-research/mice-and-rats/ [https://perma.cc/B7UN-J4MF]. The Humane Society of the United States, which advocates for an eventual end to the harmful use of animals in research, estimates that mice and rats comprise eighty-five to ninety percent of vertebrates used in research. *Questions and Answers About Biomedical Research*, HUMANE SOC'Y U.S., https://www.humanesociety.org/resources/questions-and-answers-about-biomedical-research [https://perma.cc/FJG3-YHLR].

62. SCIENCE, MEDICINE, AND ANIMALS, *supra* note 60, at 32.

63. *Accreditation*, AAALAC INT'L, https://www.aaalac.org/accreditation/steps.cfm [https://perma.cc/MJ48-Z9SY].

64. *Id.*

65. GUIDE FOR CARE AND USE, *supra* note 58; *Accreditation*, *supra* note 63.

III. ANIMAL MORAL STATUS IN U.S. AND E.U. REGULATORY GUIDANCE

The U.S. and E.U. regulatory structures guiding animal research are similar in terms of rigor and overall aim—protection of animal welfare within the constraints introduced by the needs of science. However, these systems differ in ways that are significant, at least in principle, for considering the moral status of animals in a research setting.[66] The difference between the U.S. and E.U. regulatory structures regarding animal moral status is not explicitly addressed in either set of regulatory guidance. Regulations in the United States demonstrate that some particularly harmful research, such as exposure to pathogens or radiation, is permissible in animals because "it would be unethical to deliberately expose healthy human volunteers."[67] Other than by this implied lower moral status in comparison to human subjects, however, the U.S. laws and policies generally avoid reference to the moral standing of animals used in research. What then are the grounds for stating that the two regulatory structures have different animal moral status implications? Here we briefly note four bases for this claim: first is the regulatory language describing the value of animals; second is the E.U. requirement for a harm-benefit analysis for protocol approval; third is the E.U. requirement that an upper limit be placed on pain and distress for research animals; and fourth is the particular protection the United States affords to certain species.

A. Regulatory Language Describing the Value of Animals

Regulators presumably take guidance on the moral status of research animals from institutional mandates that reflect their government's values or priorities. In the United States, the Guide requires that "all who care for, use, or produce animals for research, testing, or teaching must assume responsibility for their well-being."[68] While this sounds like a promotion of animal moral status, it is

66. By "moral status" this Article means consideration owed by ethical agents to "the needs, interests, or well-being" of animals by virtue of the kinds of creatures they are. *See* MARY ANNE WARREN, MORAL STATUS: OBLIGATIONS TO PERSONS AND OTHER LIVING THINGS 3 (1997).

67. 21 C.F.R. § 314.600 (2018). There is nevertheless a long history of exposing human subjects to very harmful pathogens, including smallpox and yellow fever. SUSAN E. LEDERER, SUBJECTED TO SCIENCE: HUMAN EXPERIMENTATION IN AMERICA BEFORE THE SECOND WORLD WAR 4, 19–21 (1995).

68. GUIDE FOR CARE AND USE, *supra* note 58, at 1.

important to note that assuming responsibility for animal well-being is compatible with a view that does not grant animals direct moral status. That is because animals whose well-being is supported are generally better sources of reliable scientific data.[69] Thus, the goal of assuming such responsibility may simply be promoting the goals of the science rather than the direct value of the animals themselves. By contrast, the E.U. framework directly recognizes that "[a]nimals have an intrinsic value which must be respected."[70] The European Union's notion of intrinsic value cannot be mistaken for the animal's instrumental value, thus going beyond the moral status implications of the U.S. guidance.

B. The E.U.'s Harm-Benefit Analysis Requirement

Specific features of the E.U. oversight that are not mirrored in the U.S. system further concretize a difference in the moral status each system grants to animals used in biomedical research. First, there is a specific requirement in the European Union for a harm-benefit analysis of research protocols before they can be approved.[71] These harm-benefit analyses do not operate on a philosophical utilitarian model of giving *equal* consideration to like interests regardless of species.[72] However, the requirement for such an analysis itself supports the idea that harms to animals must be balanced to some determinate degree against potential human (or other animal) benefit, and an ethical proposal must be able to legitimately claim that the benefits will outweigh the harms to a reasonable degree.[73] In any given system of harm-benefit calculation, the extent to which animal harms are discounted relative to human benefit exposes an unequal weight given to their ethical consideration. Whether or how this can be justified is a matter for considerable philosophical debate.[74] Nevertheless, the requirement to conduct a harm-benefit analysis itself is a signal of the European Union taking animal moral value seriously, whether discounted relative to human value or not.

69. *See* Carbone, *supra* note 9, at 1–5.

70. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 12, at 34.

71. *Id.* art. 38(2)(d), at 47.

72. PETER SINGER, ANIMAL LIBERATION 20–21 (Pimlico, 2d ed. 1995) (1990).

73. *See* Council Directive 2010/63, *supra* note 9, art. 38, at 46–47.

74. *See* Rebecca L. Walker & Nancy M. P. King, *Biodefense Research and the U.S. Regulatory Structure: Whither Nonhuman Primate Moral Standing?*, 21 KENNEDY INST. ETHICS J. 277, 277–310 (2011).

In the United States, the weighing of animal harm against potential human benefit is not similarly prioritized. The U.S. Government Principles contain the general admonition that "[p]rocedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society."[75] However, these vague appeals to scientific relevance are not carried through in any concrete requirement for a harm-benefit analysis within the IACUC protocol assessment.[76] While the process of undergoing review for National Institutes of Health ("NIH") grant funding does include rigorous assessment by both a peer group of scientists and an advisory council that includes public representation, that process also does not explicitly balance potential benefits against the harms to animals required to achieve those benefits. Thus, unlike in human-subject ethics review,[77] in the United States animal subject protocols are not necessarily assessed for their likely net value.

C. The E.U.'s Upper Pain Threshold

A third difference between the U.S. and E.U. systems is that the United States lacks an upper limit to admissible pain or distress of animals used in scientific research. In the United States, there are significant requirements that researchers "avoid or minimize discomfort" and distress to animals.[78] In particular, the requirements apply to the use of analgesics when animals will experience pain and euthanizing animals when needed as a humane endpoint.[79] However, these requirements are circumscribed by the needs of science. Accordingly, if a scientific justification can be given to withhold analgesics or to cause severe pain or distress, the science takes precedence. This could occur when pain itself is being studied so that animal suffering is mandatory to achieve the study objective. For example, a study of the potential efficacy of new pain drugs requires pain to be inflicted on animal subjects to test whether the drug alleviated that pain. The same reasoning also applies if offering pain

75. U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, 50 Fed. Reg. 20,864, 20,864 (May 20, 1985).

76. *See* Larry Carbone, *Justification for the Use of Animals*, *in* THE IACUC HANDBOOK 211, 211–36 (Jerald Silverman, Mark A. Suckow & Sreekant Murthy eds., 3d ed. 2014).

77. *See* 48 C.F.R. §§ 1352.235–70 to .235–73 (2018) (outlining the review process for research involving human subjects).

78. 9 C.F.R. § 2.31(d) (2018).

79. *Id.* § 2.31(d)(i), (iv)–(v); PHS POLICY, *supra* note 48, at 4.

relief would interfere with the data being collected and thus the validity of the study,[80] such as when the use of analgesics is avoided in animal studies because analgesics alter the animal's physiological response.[81] The IACUC review, then, must either ensure that appropriate analgesics are employed when procedures cause more than minimal pain or otherwise approve a scientific justification for withholding such pain relief.[82] An upper limit on permissible animal suffering[83] offers an ethical limitation on what may be done to animals for the sake of human benefit and suggests, in principle, that animals have some determinate moral status. Alternatively, if permissible pain and suffering is conditioned by the needs of science, where the *ethical* value of the scientific intervention is not independently assessed, then arguably animals are deprived of a moral status in exchange for their rote scientific value. It is important to note that the European Union also allows requirements for analgesia to be overridden by scientific justification.[84] The difference is in the upper limit of allowable pain established in the E.U. framework.

D. The Three Rs in Practice

As noted above, the internationally recognized ethical framework supporting and guiding animal research is the three Rs: reduction, refinement, and replacement. The contrasting approaches to the three Rs between the U.S. and E.U. systems raise somewhat more vague implications for animal moral standing. These "principles of humane experimental technique"[85] are central to the European Union's commitment to diminishing a reliance on animal research

80. Jerrold Tannenbaum, *Ethics and Pain Research in Animals*, 40 INST. FOR LABORATORY ANIMAL RES. J. 97, 97–110 (1999).

81. Carbone, *supra* note 9, at 6.

82. 9 C.F.R. § 2.31(d)(iv)(A) (2018); *see also* Alicia Z. Karas & Jerald Silverman, *Pain and Distress*, *in* THE IACUC HANDBOOK, *supra* note 76, at 317, 326–27.

83. The E.U. Directive 2010/63 remains silent regarding the specific threshold of permissible pain and suffering. However, the Directive lists in Annex VIII several procedures classified as "severe" (i.e., not reaching the upper limit and so still permissible), particularly "(a) toxicity testing where death is the end-point, . . . (b) testing of device where failure may cause severe pain, distress or death of the animal (e.g., cardiac assist devices); . . . [and] (m) forced swim or exercise tests with exhaustion as the end-point." Council Directive 2010/63, *supra* note 9, annex VIII, sec. III(3), at 79. There is thus a question about the practical significance of an "upper limit" to pain in terms of animal protection. Nevertheless, having such a limit established in law does not offer a conceptual framework that allows for the extension of direct moral standing to animals used in research.

84. *Id.* art. 14, at 42.

85. RUSSELL & BURCH, *supra* note 4, at 64.

overall.[86] In the United States, the three Rs have been implicit in the regulatory guidance but only explicitly mentioned in the more recent versions of the Guide as a "practical strategy for decision making" regarding the use of animals.[87] Moreover, there generally appears to be less commitment in the U.S. system to the idea that animal use in science is provisional. While E.U. Directive 2010/63 states that the use of live animals "*continues* to be necessary,"[88] the U.S. Government Principles simply state that "[t]he development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals *requires in vivo* experimentation with a wide variety of animal species."[89] Further, in the United States, the use of alternatives such as "mathematical models, computer simulation, and *in vitro* biological systems should be *considered*" but are not mandated.[90] While the differences between the two jurisdictions' emphasis on the three Rs are a matter of interpretation, it is significant that the European Union generally puts greater emphasis on the provisional nature of the use of animals in research. This relative focus on the three Rs in the European Union supports, at least in principle, the view that animals are ethically valuable and that their use is a moral problem that may be solved by phasing out that use over time.[91]

E. U.S. Species-Specific Protections

In addition to the differences between the U.S. and E.U. systems relative to the general moral status of research animals, the U.S. system has its own set of species-specific protections that are worth noting. As noted above, the AWA only covers certain species of warm-blooded vertebrate animals used in research. In current practice, mice in particular are widely subject to gene editing, and these animals are not covered by the AWA. Within the AWA there is specific mention of a requirement for "exercise of dogs" and "for a physical environment adequate to promote the psychological well-

86. *See* Council Directive 2010/63, *supra* note 9, pmbl. ¶¶ 10, 39, art. 1, at 34, 36, 38–39.

87. GUIDE FOR CARE AND USE, *supra* note 58, at 3.

88. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 10, at 34 (emphasis added).

89. U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, 50 Fed. Reg. 20,864, 20,864 (May 20, 1985) (emphasis added).

90. *Id.*

91. *See* Council Directive 2010/63, *supra* note 9, pmbl. ¶ 11, at 34.

being of primates."[92] Thus, both dogs and primates receive special protections under the AWA. Other than these special protections, the AWA and attending regulations generally require keeping individual records for dogs and cats, as well as for primates acquired and used in research,[93] and dictate the need for species-appropriate living conditions that contribute to the animals' "health and comfort."[94] Both the AWA and PHS Policy regulations and guidance generally require that there be a scientific justification for the appropriateness of the species used in a protocol.[95] Finally, best practices for laboratory animal euthanasia depend on the particular species.[96]

IV. IMPLICATIONS OF U.S.-E.U. REGULATORY SYSTEM DIFFERENCES FOR THE INCREASED USE OF GENE-EDITED LARGE-ANIMAL MODELS

While the United States and European Union are similar in terms of their rigorous and extensive oversight of animal research practices—and are among the most thorough oversight systems in the world—this Article has noted both practical and conceptual differences in how the systems are structured. How do these differences impact the ethics and policy implications of a greater use of gene-edited large-animal models? It may appear initially that the E.U. system is more likely to place hurdles in the way of such use due to its greater emphasis on animal moral status and the three Rs. However, as we shall argue, that is not necessarily the case.

In this part, we first spell out in further detail why we believe the three Rs cannot offer conclusive guidance regarding an increased use of gene-edited large-animal models. We then proceed by going step-by-step through the E.U. project evaluation process to consider where it might offer guidance on the issue of gene modification of large-animal models. Next, we consider how the upper limit on pain in the E.U. regulations engages the issue. Finally, we consider what species-specific protections in the United States and in the European Union

92. 7 U.S.C. § 2143(a)(2)(B) (2012).

93. *See* Angela M. Mexas & Diane J. Gaertner, *Amending IACUC Protocols*, *in* THE IACUC HANDBOOK, *supra* note 76, at 177, 192.

94. 9 C.F.R. § 2.31(d)(iv)(A) (2018).

95. *See* Mexas & Gaertner, *supra* note 93, at 181.

96. *See* AM. VETERINARY MED. ASS'N, AVMA GUIDELINES FOR THE EUTHANASIA OF ANIMALS: 2013 EDITION 48–51 (2013), https://www.avma.org/KB/Policies/Documents/euthanasia.pdf [https://perma.cc/E8H8-4G7G].

may offer. Through this process, we show why neither system inhibits a move to greater use of gene-edited large-animal models.

The three Rs, as the ethical framework underlying the responsible conduct of animal research, contain internal conflict. The conflict that is crucial for this Article, as previously discussed, is primarily between reduction and relative replacement. However, there are additional tensions within the three Rs, as well as conflicting interpretations of the individual Rs. Reduction, for example, is understood by animal protectionists as reduction of the total number of animals used in research.[97] However, those promoting animal research understand the mandate as an increase in efficiency by using the fewest animals needed for individual protocols and/or increasing information gained from each animal.[98] This second interpretation also raises the potential of reusing animals to further the aim of reduction.[99] Such reuse, however, may be in conflict with refinement, which aims to lessen the harm to each individual animal.[100] These conflicts in balancing and understanding the three Rs are significant because, ultimately, the tension between reduction and relative replacement in using large numbers of small animals versus small numbers of large animals is a tension that the professional ethical framework of animal research is incapable or unwilling to resolve. This ethical tension is thus reflected in the lack of guidance within the regulatory structures.

Animal use in the European Union is, in coordination with the three Rs, regulated by a prospective project evaluation. Does a project evaluation (which, among other items, entails a mandatory harm-benefit analysis) give guidance on how to resolve this tension between reduction and relative replacement that is apparent in an increased use of genetically modified large animals? The European Union's initial project evaluation procedure requires affirming that a project is justified from a scientific or educational point of view or required by law and that the project's purpose justifies the use of animals.[101] Moreover, the purpose of the experiment has to fall within

97. *See* I. Anna S. Olsson et al., *The 3Rs Principle – Mind the Ethical Gap!*, *in* PROCEEDINGS OF THE 8TH WORLD CONGRESS ON ALTERNATIVES AND ANIMAL USE IN THE LIFE SCIENCES, MONTREAL 2011, at 333, 333–34 (2012).

98. *Id.*

99. Council Directive 2010/63, *supra* note 9, art. 16, at 42.

100. M.J. de Boo et al., *The Interplay Between Replacement, Reduction and Refinement: Considerations Where the Three Rs Interact*, 14 ANIMAL WELFARE 327, 328 (2005).

101. Project evaluation is mainly regulated according to Article 38 in the E.U. Directive 2010/63. *See* Council Directive 2010/63, *supra* note 9, art. 38, at 46–47.

the listed legal purposes for animal use, such as basic or translational research, education, or regulatory tests, among others.[102] This step is primarily a scientific evaluation of the relevance of the research goal and whether the research design is appropriate to acquire the knowledge sought.

Next, the project must demonstrate compliance with the three Rs. According to the European Union, the species with the lowest capacity to suffer should be used, but emphasis remains on guaranteeing the optimal potential for translation of the results into the target species—most often humans.[103] If the knowledge gained from rodent studies can sufficiently be translated to the human condition, there would seem to be no justification to use larger animals in their stead. However, the low translatability of research findings has partially been attributed to the limitations of rodents as models for human diseases.[104] For this reason, the species with the lowest capacity to suffer that also brings about the most satisfactory results might not be rodents but larger animals such as dogs, pigs, or NHPs. Based on this logic, *if* the only satisfactory results can be obtained from large-animal models, and *if* any animals are used, then only these models should be used.

The next requirement in project evaluation is a severity assessment of the procedures involved and a harm-benefit analysis to weigh the interests of animals.[105] A harm-benefit analysis relies on a modified utilitarian framework (i.e., one that discounts to some extent animal welfare in relation to human welfare), according to which even significant harms to animals are justifiable as long as the expected benefit is large enough.[106] The specific language of the E.U. Directive 2010/63 calls for "taking into account ethical considerations"[107] in balancing harms and benefits; however, what exactly "ethical consideration" means in this context remains

102. *Id.* art. 5, at 40.

103. *See id.* pmbl. ¶ 13, at 34; *id.* art. 13, at 42 (expressing preference for procedures which "(a) use the minimum number of animals; (b) involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm; (c) cause the least pain, suffering, distress or lasting harm; and are most likely to provide satisfactory results").

104. van der Worp et al., *supra* note 18, at 5 ("[T]he translation of [rodent study] results to the clinic may fail because of disparities between the model and the clinical trials testing the treatment strategy.").

105. Council Directive 2010/63, *supra* note 9, art. 38(2)(d), at 47.

106. *Id.* annex VIII, sec. III(3), at 79.

107. *Id.* art. 38(2)(d), at 47.

elusive.[108] Nevertheless, there are some indications regarding ethical considerations in the E.U.'s regulatory guidance. First, as discussed above, it is linked to the notion that all sentient animals have intrinsic value that must be respected and thus their use should be restricted to areas that may ultimately benefit humans, animals, or the environment.[109] Second, the use of animals in research is only morally justified if the potential gain is sufficiently important.[110] Yet the concept of ethical guidance in the E.U. Directive 2010/63 gives no specification at all on how to weigh the use of one species over another within a harm-benefit analysis. Thus, we must extrapolate.

The potential for the generation of better (i.e., more translatable) large-animal models represents a potentially significant gain on the benefit side within the harm-benefit analysis as we have already addressed. However, since large-animal models are typically also more cognitively developed, their complex needs and interests call for a focus on refinement to induce as little suffering as possible through accommodating their special housing needs, providing adequate stimuli, and facilitating appropriate social interaction.[111] Further, hindrances to the specific social, psychological, and accommodation needs of these animals should be taken into consideration as harms of research in addition to those of the experimental intervention.[112] Overall, the potential for increased suffering of large animals used in research must be outweighed by both the potential increase in benefit to humans, animals, or the environment, as well as the reduced numbers of animals used. In short, using a harm-benefit analysis, it is possible that genetically modifying large-animal models will increase individual animal

108. *See* Herwig Grimm, *Ethics Within Legal Limits: Harm-Benefit Analysis According to the Directive 2010/63/EU*, *in* KNOW YOUR FOOD: FOOD ETHICS AND INNOVATION 42, 42–46 (Diana Elena Dumitras, Ionel Mugurel Jitea & Stef Aerts eds., 2015).

109. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 12, at 34.

110. *Id.* pmbl. ¶ 39, at 36.

111. *See* Anna Catarina Vieira de Castro & I. Anna S. Olsson, *Does the Goal Justify the Methods? Harm and Benefit in Neuroscience Research Using Animals*, *in* CURRENT TOPICS IN BEHAVIORAL NEUROSCIENCES 47, 57–63 (Grace Lee, Judy Illes & Frauke Ohl eds., 2015).

112. The use of cognitively higher-developed animals requires appropriate measures to be taken to ensure species-appropriate housing and behavior. *See id.* at 60–62. In particular, the European Union requires: "(a) all animals are provided with accommodation, an environment, food, water and care which are appropriate to their health and well-being; [and] (b) any restrictions on the extent to which an animal can satisfy its physiological and ethological needs are kept to a minimum." Council Directive 2010/63, *supra* note 9, art. 33, at 45–46.

suffering; however, under the assumption that fewer animals are used and that more effective animal research would result, absolute suffering could still be reduced. Based on the logic of the harm-benefit analysis, then, the fact that cognitively higher-developed animals may have a larger capacity to suffer than rodents does not disqualify them from use in animal research as long as the harm inflicted on them is outweighed by the benefit.[113]

Another article of the E.U. Directive 2010/63 that warrants discussion regarding the use of gene-edited large-animal models is the establishment of an upper limit on pain and distress in biomedical research. Preamble 23 of the E.U. Directive 2010/63 states as follows:

> From an ethical standpoint, there should be an upper limit of pain, suffering and distress above which animals should not be subjected in scientific procedures. To that end, the performance of procedures that result in severe pain, suffering or distress, which is likely to be long-lasting and cannot be ameliorated, should be prohibited.[114]

The concept of an upper limit on acceptable pain is interesting with regard to the belief that cognitively higher-developed animals have an increased ability to suffer. Depending on where that upper limit is, it is conceivable that higher-developed animals are more prone to reach that threshold compared to rodents. However, dogs, pigs, and NHP are used in harmful biomedical research, implying that regulators do not consider their use to automatically reach that threshold. Yet there is a legitimate concern that some animal models with genetic disorders such as "Huntington's disease, Muscular dystrophy, [and] chronic relapsing neuritis" are expected to experience severe and persistent suffering.[115] With regard to genetically modified large-animal models for specific devastating human diseases, then, it would

113. It is interesting to note, despite this Article's analysis, that a recent AALAS-FELASA working group publication on harm-benefit analysis considered the use of eighty-seven pigs for a study to be of much greater harm compared to a study that used nine hundred mice. *See generally* Kathy Laber et al., *Recommendations for Addressing Harm–Benefit Analysis and Implementation in Ethical Evaluation – Report from the AALAS–FELASA Working Group on Harm–Benefit Analysis – Part 2*, 50 LABORATORY ANIMALS (SUPP. 1) 21, 21–42 (2016). The implication from this assessment is that the trend toward use of larger animals may, in practice, only be encouraged if the benefit is significantly higher compared to the benefit of a study in smaller animals. There clearly is also a *perceived* higher ethical barrier to using large-animal models even though no specific guidance to this effect is available in the E.U. Directive 2010/63.

114. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 23, at 35.

115. *Id.* annex VIII, sec. III(3)(h), at 79.

need to be decided on a case-by-case basis whether this upper pain threshold is reached.[116]

Thus, the upper limit on pain in animal studies does not appear to rule out the use of gene-edited large-animal models, even potentially for quite devastating human diseases. It is telling from an animal moral status point of view that the U.S. regulatory structure requires neither a specific harm-benefit analysis nor suggests an upper limit on admissible pain and suffering. Yet as illustrated here, the project evaluation in the European Union seems to place few hurdles to the use of gene-edited large animals even with these safeguards in place. Thus, nothing in our analysis of either the E.U. or U.S. regulatory structures would inhibit moving to gene-edited large-animal models as a favored option to tackle intransigent human diseases.

Only the species-specific protections in each set of regulations remain as potential barriers. E.U. Directive 2010/63 requires that great apes should only be used in research under exceptional circumstances, such as preservation of the species and only for human interests under life-threatening circumstances.[117] In the United States, protections for chimpanzees in particular have evolved as a matter of institutional decisionmaking on the part of the NIH, culminating in a 2015 determination to no longer fund chimpanzee research.[118] The U.S. Fish and Wildlife Service determined in that same year that chimpanzees are an endangered species.[119] Thus, while chimpanzee research is not illegal in the United States, hurdles to performing such research are extremely high.

116. What actually constitutes the upper limit threshold is never made explicit in the E.U. Directive 2010/63. *See, e.g.*, *id.* annex VIII, at 76–79 (listing "[s]everity categories" of pain but not an upper limit). Severe suffering is still considered within an acceptable range, and a few examples of "severe" suffering include "breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example, Huntington's disease, Muscular dystrophy, chronic relapsing neuritis models." *Id.* annex VIII, sec. III(3)(h), at 79. Other nongenetic examples include "inescapable electric shock (e.g. to produce learned helplessness)" or "complete isolation for prolonged periods of social species e.g. dogs and non-human primates." *Id.* annex VIII, sec. III(3)(k), at 79.

117. *Id.* pmbl. ¶ 18, at 35.

118. Francis S. Collins, *NIH Will No Longer Support Biomedical Research on Chimpanzees*, NAT'L INSTITUTES HEALTH (Nov. 17, 2015), https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-will-no-longer-support-biomedical-research-chimpanzees [https://perma.cc/9328-P9JE].

119. Endangered and Threatened Wildlife and Plants; Listing All Chimpanzees as Endangered Species, 80 Fed. Reg., 34,500, 34,500 (June 16, 2015) (codified at 50 C.F.R. pt. 17).

Regarding the use of other NHPs, E.U. Directive 2010/63 notes both the continuing "necessity" of their use and also notes that such use raises "specific ethical and practical problems in terms of meeting their behavioural, environmental, and social needs in a laboratory environment."[120] These problems, along with social concern about the use of NHPs, leads to restrictions on their use to basic research, species preservation, or in relation to life-threatening or debilitating human conditions.[121] Importantly, E.U. Directive 2010/63 specifically states that there needs to be "scientific justification to the effect that the purpose of the procedure cannot be achieved by the use of species other than non-human primates."[122] Thus, there appears to be an effort to keep NHPs as a "last resort" model to address human disease and disability. However, given the good fit of NHPs as models for some human disease conditions, along with the fact that those diseases are frequently debilitating,[123] there is good reason to think many of the uses of gene-edited NHPs may be approved under these restrictions.

While the U.S. regulations also require species-specific justification for the use of animals in scientific research and also

120. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 17, at 34.

121. The E.U. Directive 2010/63 preamble states in full:

> Having regard to the present state of scientific knowledge, the use of non-human primates in scientific procedures is still necessary in biomedical research. Due to their genetic proximity to human beings and to their highly developed social skills, the use of non-human primates in scientific procedures raises specific ethical and practical problems in terms of meeting their behavioural, environmental and social needs in a laboratory environment. Furthermore, the use of non-human primates is of the greatest concern to the public. Therefore the use of non-human primates should be permitted only in those biomedical areas essential for the benefit of human beings, for which no other alternative replacement methods are yet available. Their use should be permitted only for basic research, the preservation of the respective non-human primate species or when the work, including xenotransplantation, is carried out in relation to potentially life-threatening conditions in humans or in relation to cases having a substantial impact on a person's day-to-day functioning, i.e. debilitating conditions.

Id. pmbl. ¶ 17, at 34–35.

122. *Id.* art. 8, at 40. Although here it is important to note that the safeguard clause also allows the use of NHPs by an E.U. member "where the use is not undertaken with a view to the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions, [the E.U. member] may adopt a provisional measure allowing such use, provided the purpose cannot be achieved by the use of species other than non-human primates." *Id.* art. 55, at 50.

123. Such debilitating diseases that could be modeled by NHP may include, for example, Huntington's disease, Parkinson's disease, Alzheimer's disease, and many others.

embrace the idea that the species with the least capacity to suffer ought to be used,[124] these regulations neither require a special justification for the use of NHPs nor restrict their use to the particular purposes outlined by E.U. Directive 2010/63. In the United States, by contrast, the most apparent oversight difference between the use of dogs, pigs, primates, other large animals, and rodents is in the lack of coverage of rodents by the AWA.[125] Practically, this means a lack of unannounced site inspections by USDA officers, a lack of reporting requirements regarding pain categories and subject numbers for rodents, and the absence of some particular requirements (such as evidence of a search for alternatives to the use of live animals).[126] As noted, the other special protections that the AWA grants in particular to dogs and primates are for exercise for dogs and attention to psychological well-being for primates. Both of these protections are in regard to appropriate housing and caretaking and neither give any special reason to think that gene-editing interventions would contravene them.

In sum, even though cats, dogs, and NHPs have specific standing in E.U. Directive 2010/63, as well as in the U.S. regulatory system, their use for scientific purposes is nonetheless allowed and only the use of NHPs is explicitly restricted in the European Union.[127] Pigs do not receive special protection in either regulatory framework, though they are covered by the AWA.[128] Against the background of the translatability crisis of animal research in general and rodent studies in particular, E.U. Directive 2010/63 does not, based on our analysis, present reasons to oppose a turn toward using cognitively higher-developed animals over cognitively less-developed animals, as long as a sufficient scientific rationale for the experiment is given. As nothing in the U.S. regulations overall offers greater levels of protection of large animals, there is no reason to think any greater resistance to gene-edited large-animal models would occur in that setting.

124. *See supra* Section III.E.

125. 7 U.S.C. § 2132(g) (2012).

126. SCIENCE, MEDICINE, AND ANIMALS, *supra* note 60, at 30; Karas & Silverman, *supra* note 82, at 339–40; Ernest D. Prentice, Gwenn S. F. Oki & Michael D. Mann, *General Concepts of Protocol Review*, *in* THE IACUC HANDBOOK, *supra* note 76, at 139, 139–40.

127. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 33, at 36 ("Non-human primates, dogs and cats should have a personal history file from birth covering their lifetimes in order to be able to receive the care, accommodation and treatment that meet their individual needs and characteristics.").

128. § 2132(g).

V. POTENTIAL IMPROVEMENTS TO THE U.S. AND E.U. FRAMEWORKS

We have identified limited areas in which the gene editing of large-animal models will not be considered ethically viable under the regulations, primarily within the E.U. legislation. Examples include the use of NHPs that are not within the approved purposes of research or for which animals other than NHPs can be used with equal efficacy. Also, unacceptable uses will be found where the perceived research benefits do not outweigh the animal harm or where the animals will suffer beyond a very high maximal threshold (as might be the case for certain devastating genetic conditions). Further, it is clear from both U.S. and E.U. regulatory standards that large animals require species-appropriate housing, caretaking, and "enrichment,"[129] as well as species-specific euthanasia methods.[130] However, these requirements are not specific to gene-edited animals, and since all large-animal species that are likely to be used for gene-edited purposes are already in use in invasive biomedical research, these constraints likely will not limit their use for gene-editing purposes.

In a sense, these conclusions are not surprising given that neither the U.S. nor the E.U. regulatory framework, despite being among the most stringent in the world, aims to manage overall trends in animal research from an ethical viewpoint. Indeed, neither contains any means to critically assess such trends. At the same time, as we have also pointed out, there are important differences between the two regulatory frameworks such that it is at least arguable that the E.U. framework grants research animals, in general, a higher moral status than in the U.S. framework. It may seem to follow from a greater emphasis on animal moral status that animals capable of greater suffering and more complex social, cognitive, and emotional capacities will be protected from highly invasive and/or harmful experiments. However, as we have discussed, even NHPs do not seem protected against being used in larger numbers to better model devastating human diseases in either regulatory framework.

One conclusion could be that the higher moral status of research animals in the European Union has little significant practical importance in terms of the protection of research animals. Yet certain types of NHP research have received added scrutiny and have even

129. Council Directive 2010/63, *supra* note 9, annex III, sec. A(3.3)(b), at 56.

130. *See* AM. VETERINARY MED. ASS'N, *supra* note 96, at 6–7.

been halted in the European Union and in Switzerland. In Bremen, Germany, for example, the license of a macaque researcher was not renewed because his work involving fixation of macaques in the primate chair to record their brains while performing simple tasks was deemed to be "too far from applications" and because "it is ethically not justifiable to inflict this kind of pain on animals for the generation of neurobiological basic knowledge."[131] However, the highest court in Germany later overturned this refusal to renew the project license.[132] A similar case happened in 2006 in Zurich when authorities declined to renew a license for primate work.[133] The authorities ruled that the work, which had a goal to map the functional microcircuitry of the brain of macaques, offended the dignity of the animals and would not generate practical benefits in the foreseeable future.[134] Decisions like these imply that a greater use of large-animal models may be perceived as justified only as long as the promise of translational efficiency is salient to those reviewing the research.

While there are no features of the current regulatory guidance in the United States or the European Union that we identified as necessarily inhibiting a trend to greater use of gene-edited large-animal models, there are broader ethical implications of such a trend that are critical to considering its overall acceptability. We raise these briefly here but note that they deserve much greater attention than we give them in the context of this Article, focused as it is on the regulatory implications of such a trend.

One set of issues has to do with whether some genetic manipulations, for example brain enhancements, might themselves incur greater sentience and thus a more significant ability to suffer in large animals. This is a particularly salient concern for NHPs, whose

131. Herwig Grimm et al., *The Road to Hell Is Paved with Good Intentions: Why Harm-Benefit Analysis and Its Emphasis on Practical Benefit Jeopardizes the Credibility of Research*, 7 ANIMALS, no. 7090070, Sept. 11, 2017, at 1, 2 (translating *Oberverwaltungsgericht der Freien Hansestadt Bremen*, FREIE HANSESTADT BREMEN (Dec. 11, 2012), https://www.oberverwaltungsgericht.bremen.de/sixcms/detail.php?gsid=bremen72.c.11099.de&asl=bremen72.c.11265 [https://perma.cc/V2BK-DMJA]); *see also* Quirin Schiermeier, *German Authority Halts Primate Work*, 455 NATURE 1159, 1159 (2008).

132. Hristio Boytchev, *Campaign Targeting Animal Experimenter Causes Uproar in Germany*, SCIENCE (May 7, 2014, 4:45 PM), https://www.sciencemag.org/news/2014/05/campaign-targeting-animal-experimenter-causes-uproar-germany [https://perma.cc/JQ4M-HJXH].

133. Alison Abbot, *Basel Declaration Defends Animal Research*, 468 NATURE 742, 742 (2010).

134. *Id.*

cognitive capacities are already so similar to our own. Closely tied to this concern is the critique that the moral status implications of the E.U. legislation do not go far enough. On that view, it is not enough to grant harms to animals like dogs and primates a higher weight than harms to rodents in a harm-benefit analysis; rather, these animals should be granted a moral status such that they simply are not used in harmful animal research. Of course, if that is the case, then the move to gene-edited large-animal models will be blocked out of the gate. A related, but theoretically very different, approach would be to claim that it is simply not the case that the social, emotional, and cognitive needs of these larger mammals can be adequately met within the confines of a research facility. Should this empirical claim be proven true, and under the assumption that meeting those needs is a precursor to the ethical use of any animals, it may be argued as a matter of practical necessity that such animals should not be used.

A final theoretical point falls along the lines of a utilitarian critique of the harm-benefit analysis within the E.U. regulatory framework. Opponents might argue that within the normative framework of E.U. Directive 2010/63, animal interests are discounted beyond what is ethically acceptable. This criticism is based on the fact that even the fairly remote promise of human benefit might justify quite serious actual harms to animals according to the current practices associated with harm-benefit analyses. Thus, a more balanced equitable consideration of harms and benefits will justify far less in the way of animal research generally, including the use of gene-edited large-animal models. Of course, such an argument leaves completely open the idea that some uses of gene-edited large animals could well be justified, especially if they promise particularly compelling health advances for recalcitrant human diseases.

The ethical concerns that may be raised with the greater use of gene-edited large animals in biomedical research largely fall outside the scope of the regulatory framework for animal research that we have discussed. However, there are important precursors to these potential concerns within the regulatory frameworks themselves, especially within the E.U. framework. For example, the idea of the requirement for a harm-benefit analysis as an ethical justification for animal research is a necessary step in moving toward a more equitable treatment of animal interests within such an analysis. Similarly, the idea that certain harms are out of bounds for sentient animals even if they may bear fruit scientifically is a conceptual step toward the idea that animals in research may have certain rights in regard to how they are utilized. Finally, the concern about species-

specific housing, caretaking, social needs, etc., when taken to its logical endpoint, may lead to a concern that it is potentially impossible for animal confinement facilities to adequately meet complex social, emotional, and cognitive needs of certain types of animals (even more so if those animals are cognitively enhanced by genetic means).

One way of seeing this set of issues is that, in so far as they sign onto the three Rs, and in particular to replacement as incrementally realizable, animal scientists generally agree that harmful animal use is less than ideal and that the ultimate goal ought to be ending the harmful use of sentient animals.[135] Put in that way, the question resulting from the use of greater numbers of gene-edited large animals may be a question of what means we might be willing to endorse to achieve that broader goal of ending harm to sentient animals. If gene-edited large animals are such excellent models for humans that their use will bring a quicker end to the harmful use of animals in biomedicine overall, is that grounds for those uses? Alternatively, the use of NHPs has raised concerns due to their ethically salient similarities to humans. If genetically modified NHPs are such good models of human disease (because they are even more humanlike), then their use raises concerns akin to the uses of humans for these same kinds of experiments and raises the question whether they, by genetic modification, would reach a limit on permissible use independently of potential benefit.

CONCLUSION

We have argued that neither the U.S. nor E.U. regulatory frameworks offer guidance on a trend toward greater use of gene-edited large animals in place of larger numbers of small-animal models. Moreover, we have suggested that the promise of greater gains in translation and efficiency are powerful forces that are likely to move the science community to embrace such trends in the name of a professional ethic that views animal use as justified mainly by its positive benefit. Further, the professional ethical framework governing animal research, the three Rs, itself contains internal tensions that make it incapable of offering significant guidance on this topic over and above the regulatory framework itself. This state of

135. This is reflected in the E.U. 2010/63 Directive's ultimate goal of ceasing animal experimentation as soon as adequate nonanimal alternative methods are available. Council Directive 2010/63, *supra* note 9, pmbl. ¶ 10, at 34.

affairs leads us to ask: Is the use of genetically modified large animals in order to realize the eventual goal of phasing out invasive animal research a step in the right direction? Alternatively, do increased uses of gene-edited large mammals like dogs, pigs, and NHPs undercut the very moral sensibilities that motivated this goal in the first place? This Article leaves this conundrum for our readers to consider.