THE 3RS AND NON-HUMAN ANIMALS IN BIOMEDICAL RESEARCH: THE NEXT 65 YEARS

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It has been 65 years since the publication of Russell and Burch's "The Principles of Humane Experimental Technique," which established the '3Rs'—refinement, reduction, and replacement—as the key principles applicable to decision making about, and the use of, non-human animals in laboratory settings. The 3Rs are universally accepted by responsible scientists throughout the world and form the basis for many national legal and regulatory systems governing animal use in laboratories. This Article will discuss broadly how the 3Rs have evolved over the past seven decades since the publication of Russell and Burch's seminal work, and examine the 3Rs in light of the needs of the biomedical research challenges today and into the future. The Article also evaluates recent critiques of the 3Rs and assesses whether such critiques are warranted considering current research practices and societal concerns. To make the 3Rs maximally useful for the 21st century and beyond, additional principles should be added that take into consideration (1) the need to strengthen reproducibility and predictivity of animal-based research; (2) the recognition that animals are sentient beings whose basic needs must be met; and (3) the importance of a harm-benefit evaluation that addresses the benefits, risks, and harms to humans as well as animals. The 3Rs must evolve in these ways so that science can continue to develop, and laboratory animal use can be reduced or replaced.

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I. INTRODUCTION

It has been sixty-five years since Russell and Burch published *The Principles of Humane Experimental Technique*, which established the '3Rs'—refinement, reduction and replacement—as the key principles applicable to decision making about, and the use of, non-human animals in laboratory settings.[1] The 3Rs are universally accepted by responsible scientists throughout the world and form the basis for many national legal and regulatory systems governing animal use in laboratories. This Article will discuss the current state of the 3Rs, their non-incorporation into U.S. federal law, and describe how the 3Rs have evolved over the past seven decades since the publication of Russell and Burch's seminal work.

Part II of this Article introduces the 3Rs in detail, focusing on Russell and Burch's treatise and its implications for laboratory animals and research. Part III examines the relationship between the 3Rs and U.S. federal laws by reviewing the development of the Animal Welfare Act, the Health Research Extension Act, and associated policies and regulations. It also charts the uptake of the 3Rs in practice. In Part IV, this Article examines some of the criticisms of the 3Rs and proposals for changes in the 3Rs to align them with more modern science and ethics. Part IV also points out the rise of, and challenges associated with, the incorporation of non-animal models in toxicology practice. Part V concludes by applying earlier analysis to make suggestions about how the 3Rs should be improved.

II. THE GENESIS OF THE 3RS

In 1954, Dr. William Russell and Mr. Rex Burch were commissioned by the Universities Federation of Animal Welfare to undertake a systematic study of laboratory techniques involving non-human animals, from an ethical perspective.[2] This study was deemed necessary because

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[1] W.M.S. RUSSELL & R.L BURCH, THE PRINCIPLES OF HUMANE EXPERIMENTAL TECHNIQUES 64 (Special Edition) (1992).

[2] W.M.S. Russell & R.L. Burch, *Foreword to Special Edition* to THE PRINCIPLES OF HUMANE EXPERIMENTAL TECHNIQUE (Universities Federation for Animal Welfare) (1992); *See About UFAW*, UNIV. FED'N. FOR ANIMAL WELFARE, https://www.ufaw.org.uk/ (accessed Jan. 30, 2024); *History of UFAW*, UNIV. FED'N. FOR ANIMAL WELFARE, https://www.ufaw.org.uk/

of the rapid growth in the number of animals used in research after World War II.[3] Russell and Burch couched the need for their study in both scientific and philosophical terms, noting that "we owe animal experimentation many, if not most of the benefits of modern medicine and countless advances in scientific knowledge," and that "to some extent, the wages of inhumanity were paid in ambiguous or otherwise unsatisfactory experimental results."[4]

In 1959, the results of their inquiry were published in a treatise titled *The Principles of Humane Experimental Technique.*[5] Among other things, Russell and Burch created three concepts known as the 3Rs—replacement, refinement, and reduction. This Article examines the 3Rs to assess their evolution and their applicability to current scientific research. According to Russell and Burch, they were necessary to assure robust safety testing that underpins both animal welfare and scientific merit.[6] They noted that the growth of medical, veterinary, and pharmaceutical research "brought about a vast increase in the numbers of non-human animals employed as subjects of experiments."[7]

At the outset of their treatise, Russell and Burch laid out the central proposition upon which the 3Rs are based. This proposition has become part of the scientific bedrock upon which non-human animal experimentation is based. In the words of Russell and Burch:

> It sometimes seemed that there is an irreconcilable conflict between the claims of science and medicine and those of humanity in our treatment of lower animals. When, in the late nineteenth century, this conflict appeared to come to a head, the British genius for compromise asserted itself, and the famous Cruelty to Animals Act of 1876 balanced rival claims. Even at that early date, it was to some extent apparent that the wages of inhumanity were paid in ambiguous or otherwise unsatisfactory experimental results. This conflict disappears altogether on close inspection, and *by now it is widely recognized that the humanest possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments*. Since the Second World War … this principle has been increasingly accepted; and the intimate relationship between humanity and efficiency in experimentation will recur constantly as *a major theme in the present book*.[8]

(accessed Feb. 3, 2024) (stating that the Universities Federation of Animal Welfare was established in 1926 as a UK based, internationally recognized, independent, scientific and educational animal welfare not-for-profit organization concerned with improving knowledge and understanding of animals' needs).

[3] *See* RUSSELL & BURCH, *supra note 1,* at 5. (Russell and Burch noted that from 1945 to 1954, the number of animal experiments had increased from approximately 1 million to 3 million.).

[4] *Id*. at 3.

[5] *See generally id.*

[6] The term "animal" and "non-human animal" are used interchangeably in this paper to refer to any living creature that is not human.

[7] RUSSELL & BURCH, *supra* note 1, at 3.

[8] *Id.* at 3–4 (emphasis added) (citations omitted).

Thus, at the outset of this study, the relationship between 'humaneness' and high-quality science was established. After this introductory chapter, Russell and Burch launch the substantive portion of their work with a detailed discussion about the concept of inhumanity and what it means, in practical terms, for laboratory research.[9] Starting from the assumption that "experimental biologists are only too happy to treat their animals as humanely as possible,"[10] they turn to what they describe as the central practical problems: determining what humaneness means and how to measure it. That inquiry brings them to the concepts of pain and distress and their recognition and management.

Russell and Burch next devote a chapter to discussing the nature of human and non-human animal interactions.[11] They note the pervasive way that humankind has altered the planet and point out that "[t]he problems created by our expansion are often the subject of warnings, and great efforts are beginning to be made at international levels to . . . control Man's Role in Changing the Face of the Earth."[12] After this expansive beginning, Russell and Burch address the more immediate problem for laboratory animals, which are captive and purpose-bred beings in a human created ecosystem. Focusing on the 'removal of inhumanity' approach discussed earlier in their study, they introduce (or re-introduce) the concepts of replacement, reduction, and refinement.[13]

Russell and Burch's original definitions of the 3Rs concepts were consistent with mid-twentieth-century science and thought and were based on the connection between humaneness and quality science. According to Russell and Burch:[14]

A. REPLACEMENT

Replacement was a scientific method employing non-sentient material when previously a living vertebrate was used for the experiment. Replacement is divided into two sections, relative and absolute. Absolute replacement refers to an experiment that does not require non-human animals at any stage. Relative replacement refers to experiments where

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[9] *See infra*, Part IV. (Some of the discussion in, and examples used, in Principles are based on scientific knowledge of the mid-twentieth century. Given the advances in data and techniques since then, these examples have not been discussed in this article. An important question that arises is whether these mid-twentieth century concepts are applicable to the new scientific techniques that arose since the 3Rs were first developed. For example, Russell and Burch could not have conceived of "humanized mice"—rodents that contain one or more human genes—or "knockout mice"—rodents that have had a certain segment of genetic material removed or inactivated).

[10] RUSSELL & BURCH, *supra* note 1, at 14.

[11] *Id*. at 31-53 (Chapter III discusses "Man and Animal World").

[12] *Id.* at 31. (citation omitted) (Russell and Burch link treatment and use of laboratory animals as a subset of the broader issues associated with humankind's relationship to the global ecology).

[13] *Id*. at 64.

[14] *Id*. at 69–75, 105–07, 134–35 (the following sections rely on language and terminology used by Russell & Burch in their 1959 publication).

animals are used but are not put through any distress. Examples of relative replacement include animals being painlessly euthanized before an exposure or anaesthetized during an experiment with effects that do not outlast the anaesthesia, or if the animal is anaesthetized in a 'non-recovery' experiment.[15]

B. REDUCTION

Reduction means using fewer animals in any given experiment while still obtaining scientifically robust data. In *The Principles of Humane Experimental Technique,* reduction is paired with focusing on strategy when setting up any research involving animals. Russell and Burch note that reduction paired with relative replacement and absolute replacement are the optimal options.[16]

C. REFINEMENT

Refinement has the objective to eliminate or minimize the pain and distress an animal would be exposed to in an experiment. Under refinement, experiments are broken up into two categories: stressful or neutral experiments. Stressful experiments are when the outcome or mechanisms of pain and distress are being studied. All other experiments fall into the neutral category.[17]

III. THE UPTAKE AND APPLICATION OF THE 3RS IN THE UNITED STATES

A. THE 3RS IN U.S. FEDERAL LAW

Early legislative efforts to address animals in research did not focus on the 3Rs. Other issues, such as preventing the seizure of dogs in shelters for research, occupied the attention of advocates in the 1960s.[18] The 3Rs did not gain much traction internationally, and especially in the United States, until the 1980s. While there were efforts to improve laboratory animal welfare for all research species, such efforts were marginal and did not include the widespread adoption of the 3Rs.[19] The 1966 passage of the first federal law to address laboratory animals, titled the Laboratory Animal Welfare Act,[20] did not reference or otherwise reflect the 3Rs. The Act focused largely on licensing providers of

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15 *Id*. at 69–71. (A non-recovery experiment is an experiment in which the animals used are not able to be brought back to a healthy state and are killed when the procedures are complete).

16 *Id.* at 64, 71, 105.

17 *Id*. at 64, 134.

18 ANIMAL WELFARE INSTITUTE, ANIMALS AND THEIR LEGAL RIGHTS 67 (4th ed. 1990).

19 Robert C. Hubrecht & Elizabeth Carter, *The 3Rs and Humane Experimental Technique: Implementing Change*, 9 ANIMALS 754, 758 (Sept. 30, 2019).

20 NAT'L. RSCH. COUNCIL, SCIENCE MEDICINE & ANIMALS, 29 (2004).

animals and preventing the theft of pets, establishing humane treatment standards for dealers, and registering dealers and research facilities.[21] Only research facilities that used dogs or cats were subject to these standards.[22] This law was amended in 1970, and its name was changed by dropping the word 'Laboratory' from the title.[23] This new name of the statute—the Animal Welfare Act or AWA—is how the law is generally referenced today. The law included other changes, such as increasing coverage to warm-blooded animals (with discretion given to the Secretary of Agriculture to list such species).[24] The 1970 amendment also defined research facilities.[25] In 1976, the AWA was amended again, primarily to address animal fighting.[26]

There were other efforts to address laboratory animal welfare that did not involve federal legislation. For example, in 1963 the U.S. National Institutes of Health (NIH) developed the first guide for the care and use of laboratory animals.[27] Before the passage of the 1985 amendments to the AWA and the Health Research Extension Act, this guidance document was used by researchers to direct their behavior toward more humane practices. This guide has come to play a central role in laboratory management of animal welfare issues and is incorporated by policy into the Health Research Extension Act.[28] It has been amended frequently; its latest revision was published in 2011.[29]

The 1985 amendments to the AWA are, and remain, the most important statutory changes applicable to animals in laboratories. Unlike earlier amendments, this legislation authorized the United States Department of Agriculture (USDA) to promulgate rules that directly affected animals during experimentation.[30] These amendments required six important things: (1) Minimizing pain and distress during experimentation (unless the experiment requires otherwise) by requiring the use of analgesics and anesthetics;[31] (2) Establishing an Institutional Animal Care and Use Committee (IACUC) at all facilities

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21 ELENI G. BICKELL, CONG. RSCH. SERV., R47179, THE ANIMAL WELFARE ACT: BACKGROUND AND SELECTED ISSUES SUMMARY (2023); GENEVIEVE K. CROFT, CONG. RSCH. SERV., R47180, LEGISLATIVE HISTORY OF THE ANIMAL WELFARE ACT: IN BRIEF (2022).

22 CROFT, *supra* note 21 at 1.

23 *Id*.

24 *Id*. at 1–2.

25 *Id*. at 2.

26 *Id.*

27 INST. OF LAB'Y ANIMAL RES. COMM'N ON LIFE SCI., GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS xiii (8th ed. 2011).

28 Health Research Extension Act, Pub. L. No. 99-198, 99 Stat. 820 (1985). *See* Laboratory Animal Welfare: Adoption of Eighth Edition, 76 Fed. Reg. 74803, 74803–04 (Dec. 1, 2011) (incorporating The Guide by reference through the assurance process).

29 *See* INST. OF LAB'Y ANIMAL RES. COMM'N ON LIFE SCI., *supra* note 27 (stating that the 8th edition of the Guide was copyrighted in 2011 to replace previous versions as the most up-to-date source of guidance on the topic).

30 *See* 7 U.S.C.S. §2143(a)(1); 7 U.S.C.S. §2132(b).

31 7 U.S.C.S. § 2143(a)(3)(A).

covered by the AWA to increase public confidence in decision-making;[32] (3) Requiring enrichment for non-human primates and dogs;[33] (4) Training animal care personnel, including principal investigators (PIs) in humane techniques;[34] (5) Establishing an informal service at the national agricultural library to be a resource to reduce unintended duplication of experiments, help replace and reduce animal use, and assist in minimizing pain and distress;[35] and (6) Requiring PIs to consider alternatives to any procedures likely to produce pain and distress.[36] Additionally, this legislation requires the USDA to inspect each facility once per year.[37]

The 1985 amendments focused on humane care and treatment of laboratory animals and the minimization of their pain, and established a self-regulatory body to carry out this objective. While the findings of this bill specifically reference the development of alternatives to animal experimentation, the need for public acceptance of research, and the importance of minimizing pain, the 3Rs were not directly incorporated into the AWA during its 1985 amendment.[38] Instead, limited provisions were added to the AWA regulations that delegate responsibilities to IACUCs to implement certain aspects of the 3Rs.[39] Specifically, IACUCs must review activities to ensure that procedures involving pain, discomfort, and distress are minimized, and that for procedures causing more than momentary pain and distress, principal investigators must conduct a search to evaluate whether alternatives are available.[40] Some legal scholars have opined that these provisions indicate that the 3Rs have been made part of the AWA,[41] and at least one member of Congress has also expressed this viewpoint.[42] However, a close reading of the AWA and its regulations indicates that this interpretation is not correct. While the 3Rs are incorporated into the practices of laboratory animal programs in the United States, they are not found in the AWA or its implementing regulations.[43]

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32 7 U.S.C.S. § 2132(n); 7 U.S.C.S. §2143(b)(1).

33 7 U.S.C.S. § 2143(a)(2)(B).

34 7 U.S.C.S. § 2143(d).

35 7 U.S.C.S. § 2143(e).

36 7 U.S.C.S. § 2143(a)(3)(B).

37 Food Security Act of 1985, Pub. L. No. 99-198 § 1752(7)(A)-1753, 99 Stat. 1645, 1646, 1649 (1985) .

38 Food Security Act of 1985, Pub. L. No. 99-198 § 1751(2), 1751(4), 1752(3)(A)-(B), 99 Stat. 1645, 1645–16-46, 1648–16-49 (1985).

39 IACUC, 9 C.F.R. § 2.31(c)–(d) (2023).

40 IACUC, 9 C.F.R. § 2.31(d)(i)–(ii).

41 Darian M. Ibrahim. *Reduce, Refine, Replace: The Failure of the Three R's and the Future of Animal Experimentation*, 2006 U. CHI. LEGAL F. 195, 196–99 (2006).

42 *See* 102d Cong. E8533 (1991) (Statement of Lee Hamilton, Member, State of Indiana House of Representatives *Animal Welfare Policy: Hearing on H.R. 8533 Before the H. Comm. On the Judiciary*).

43 Gilly Griffin & Paul Locke, *Comparison of the Canadian and US Laws, Regulations, Policies, and Systems of Oversight for Animals in Research*, 57 ILAR JOURNAL, 271, 273 (2016).

In 1985, Congress also enacted the Health Research Extension Act of 1985 (HREA).[44] Its provisions apply to laboratory animal research that is funded by U.S. public health agencies, and its requirements parallel the provisions of the 1985 amendments to the AWA.[45]

B. THE 3RS IN LABORATORY PRACTICE

Even though the 3Rs have not been explicitly made part of U.S. federal laws, they are widely accepted in practice.[46] The *Guide for the Care and Use of Laboratory Animals* ("the Guide") is the vehicle through which the 3Rs have obtained this role.[47]

The Guide explains the importance of the 3Rs in its first chapter. As explained in its preface, the Guide is intended to "assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles."[48] It stresses that following the 3Rs is a practical and ethical way to adhere to the principles contained in its pages.[49] According to the Guide, the 3Rs are defined as follows:

> *Replacement* refers to methods that avoid using animals. The term includes absolute replacements (i.e., replacing animals with inanimate systems such as computer programs) as well as relative replacements (i.e., replacing animals such as vertebrates with animals that are lower on the phylogenetic scale).
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> *Refinement* refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. While institutions and investigators should take all reasonable measures to eliminate pain and distress through refinement, IACUCs should understand that with some types of studies there may be either unforeseen or intended experimental outcomes that produce pain. These outcomes may or may not be eliminated based on the goals of the study.
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> *Reduction* involves strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from a given number of animals (without increasing pain or distress) so that in the long run fewer animals are needed to acquire the same scientific information. This approach relies on an analysis of experimental design, applications of newer technologies, the use of appropriate statistical methods, and control of environmentally related variability in animal housing and study areas[.][50]

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44 Health Research Extension Act of 1985, Pub. L. No. 99-158 § 495(a).

45 Health Research Extension Act of 1985, Pub. L. No. 99-158 § 495(b)-(c). (The HREA is not discussed in detail in this article because, for purposes of interpreting the 3Rs, its requirements are nearly identical to the AWA's requirements).

46 Griffin & Locke, note 43, at 273.

47 *Id.* at 274.

48 Inst. of Lab'y Animal Res. Comm'n on Life Sci., *supra* note 27.

49 *Id.* at 1, 3–4.

50 *Id*. at 5.

This definition of the 3Rs is remarkably like the Russell and Burch definition set out in their 1959 treatise. Even though they are not explicitly included in the statutory language of the HREA, the 3Rs are incorporated by reference in the two major policies that the NIH has published to implement the provisions of HREA.[51] Both of these documents reference the Guide as the key document that facilities should use when establishing their animal welfare and care programs.[52]

Private accreditation organizations, such as the American Association for Accreditation of Laboratory Animal Care (AAALAC), also use the Guide as a centerpiece for accreditation and membership.[53] The AAALAC is a private, not-for-profit organization.[54] Its goal is to promote the humane treatment of animals used in research.[55] AAALAC has established a voluntary accreditation program in pursuit of this goal.[56] Over 1,000 companies, universities, hospitals, government agencies, and other research institutions in fifty countries and regions have been accredited by AAALAC.[57] These facilities volunteer to participate in AAALAC's program in addition to complying with the local, state, and federal laws that regulate animal research.[58]

IV. THE 'MODERN' 3RS—READY FOR THE NEXT 65 YEARS?

Although uptake of the 3Rs was relatively slow in the first thirty years following their introduction, they have since become the predominant guiding principles for laboratory animal research programs. Questions about the applicability and value of the 3Rs have arisen since their adoption, in light of twenty-first century science and societal norms, and there seems to be a growing consensus that the 3Rs must evolve and adapt to meet modern trends. In addition, there is a school of thought that would move away from the 3Rs to an entirely new standard for evaluating laboratory animal use.[59]

This section introduces some of these suggested changes and seeks to address them by considering two questions. First, assuming that the 3Rs continue to be applied to laboratory animal research, how can the

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51 *See* OFF. LAB'Y ANIMAL WELFARE, NAT'L INSTS. HEALTH, PUBLIC HEALTH SERVICES POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS 4, 5, 10, 13 (2015) (detailing the HREA principles, which emphasize the need to "consider," "avoid" and "minimize" animal pain and contribute to the "health and comfort" of animals).

52 *Id.* at 4, 8.

53 *Id.* at 10, 12.

54 *What is AAALAC?*, AM. ASSOC. FOR ACCREDITATION OF LAB'Y ANIMAL CARE INT'L, https://www.aaalac.org/about/what-is-aaalac (accessed Jan. 28, 2024).

55 *Id.*

56 *Id.*

57 *Id.*

58 *Id.*

59 This article does not attempt to cover all discussion about the 3Rs that has occurred in the past sixty-five years. Instead, it focuses on what the authors believe to be some of the major approaches to adapting or replacing them.

3Rs be amended or adapted to better serve research animals and new knowledge? Second, do the 3Rs need to be replaced altogether by a new paradigm?

A. ADAPTING AND AMENDING THE 3RS

Modern scholars have questioned whether the 3Rs, which are based on a utilitarian ethical framework, should continue as the driving principles for scientific research involving non-human animals. For example, it might not be enough for the 3Rs to merely focus on the idea that better animal welfare enhances research, which is one of the central tenets of the Russell and Burch treatise.[60] Some argue that the 3Rs should be recast to emphasize the translational value of the research.[61] Concentrating on the predictive value of any animal model to the human condition seems consistent with the replacement and, in addition, maximizes the contributions of the animals used in experimentation.[62]

Graham and Prescott's view is consistent with other scientific writers, several of whom focus on the relationship between experimental validity and the harm-benefit ratio.[63] As currently conceived, the 3Rs do not assess the quality of the underlying science and its potential to contribute to knowledge. Thus, even a study with very little predictive value could be deemed to be acceptable under a current 3Rs analysis. The recent calls in the scientific literature about the need to improve scientific validity and reproducibility raise the question as to whether a new principle is needed that should include experimental strength.[64]

A harm-benefit ratio analysis has been suggested that would weigh the '3Vs' and the 3Rs.[65] These 3Vs—assessing construct, internal, and external validity—would be used alongside the 3Rs to carry out a harm-benefit analysis on potential research projects. Construct validity should be based on evidence about the level of agreement between the animal model and human variable of interest and the quality it is meant to measure.[66] Internal validity should be based on evidence for the scientific rationale and rigor in terms of measures of bias, including the use of control groups, definition of primary and secondary outcome variables, sample size calculation, randomization, blinding and statistical analysis plan.[67] External validity should be based on the experimental design features and their ability to "enhance, or facilitate

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60 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, 27 (2015).

61 *Id*.

62 *Id*.

63 *See generally* Hanno Würbel, Commentary, *More Than 3Rs: The Importance of Scientific Validity for Harm-Benefit Analysis of Animal Research,* 46 LAB ANIMAL 164 (2017).

64 *Id*. at 165.

65 *Id*.

66 *Id*.

67 *Id*.

inference about, the reproducibility and generalizability of the expected results."[68] A harm-benefit analysis would thus compare the 3Rs and the 3Vs to reach a decision about going forward with an experiment involving non-human animals.[69] According to its supporters, adding a 3V and harm-benefit analysis to the 3Rs approach would add an important gate-keeping function: research protocols that were approved based on these criteria would be much more likely to contribute to assessing and ultimately increasing scientific validity, which can help treat and prevent disease.[70] However, harm-benefit analyses and the evaluation of predictivity are much more difficult in traditional discovery research, which is incremental in nature.[71]

Beauchamp and DeGrazia provide a comprehensive discussion of the general principles of animal research ethics and the 3Rs.[72] They acknowledge that for the past six decades the major canonical text in this area has been the 3Rs, as first outlined by Russell and Burch's *Principles of Humane Experimental Technique*.[73] While pointing out the value of the 3Rs, Beauchamp and DeGrazia conclude that the 3Rs are no longer adequate.[74] They offer a new model based on six principles which they believe are the necessary conditions of morally justified research.[75] These principles are divided into two groups: principles of social benefit and principles of animal welfare.[76]

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[68] *Id.*

[69] *Id.* at 164–65.

[70] *Id.* at 165.

[71] *See* Yoram Gutfreund*, Harm-Benefit Analysis May Not Be the Best Approach to Ensure Minimal Harms and Maximal Benefits of Animal Research—Alternatives Should Be Explored*, 10 ANIMALS 1, 6 (2020), ("The difficulty arises from the nature of basic research where the benefits are manifested indirectly at a global level while the harms, if exist, are inflicted directly at the individual research level.") (stating that harm-benefit analyses are not well suited for evaluating discovery research. Discovery research almost always addresses a sub-question about, or a small piece of, a more complex question. On its own, any particular discovery experiment might provide some evidence about a pathway toward a disease endpoint. Ultimately it must be combined with other research to shed light on the process that leads to disease. In addition, discovery research rarely travels a linear and direct road. There are twists and turns that do not shed direct light on the condition of interest, but instead are valuable for ruling things out or generating new hypothesis for additional research).

[72] *See generally* David DeGrazia & Tom Beauchamp, *Beyond the 3 Rs to a More Comprehensive Framework of Principles for Animal Research Ethics,* 60 ILAR Journal 308, 308-10 (2019) (while the 3Rs "represent a landmark advance in the promotion of animal welfare and good science," there have since been "numerous social, political, and institutional developments" which have greatly increased public concern about animal research ethics).

[73] See *id*. at 309–10 ("[C]urrently available framework [includes] the influential one presented in *Principles of Humane Experimental Technique*, published in 1959 by zoologist and psychologist William M. S. Russell and microbiologist Rex L. Burch. Their principles are commonly referred to as the 3 Rs.").

[74] *Id.* at 309.

[75] *Id.* at 311.

[76] TOM L. BEAUCHAMP & DAVID DEGRAZIA, PRINCIPLES OF ANIMAL RESEARCH ETHICS 3 (2020).

Their principles of social benefit arise from the relationship between the social benefits of the research and its costs and risks of harm: (1) The principle of 'no alternative method'. Use of animals must be the sole ethically acceptable method to carry out the research and address the scientific question at hand. This inquiry must be based on a careful review of available methodologies and a close analysis of them.[77] (2) The principle of 'expected net benefits'. The social good from the research must outweigh the costs and risks to humans. Again, this decision must be based on a carefully reasoned argument.[78] (3) The principle of 'sufficient value to justify harm'. The projected net benefit of the research to humans must considered valuable enough to justify the expected harms to the laboratory animals that are part of the research. The basis for this decision must be explicitly documented.[79] For each of these three principles, the rationale should be explicitly stated and explained in the experimental protocols.

The principles of animal welfare are based on the understanding that all sentient beings and their lives have value.[80] In laboratory settings, non-human animals have certain basic needs that require attention. It is inhumane to fail to recognize, and act upon, this understanding. The principles of animal welfare are: (1) The principle of 'no unnecessary harm'. Unless morally justified by a scientific purpose, animal subjects should not be harmed.[81] (2) The principle of 'basic needs'. The basic needs of animal subjects must be met, unless failure to meet those needs is morally justified by scientific purposes.[82] (3) The principle of 'upper limits to harm'. Animals should not have to experience severe suffering for a lengthy period.[83] This principle might be waived in rare circumstances, such as if it is warranted by the research and morally justified and critically important social and scientific goals are at stake.[84]

These six principles do not conflict with the 3Rs and are meant to build on them by closing gaps, as Beauchamp and DeGrazia point out.[85] They extend beyond the 3Rs in several important ways. The 3Rs promote harm reduction, and due to the application of the 3Rs research is not deemed to be justifiable if non-animal alternatives are available, if the appropriate number of animals is not used, and if pain, stress, and distress are not addressed. However, the 3Rs do not include the need to assess social benefit, nor do they contain principles regarding comprehensive animal welfare.[86]

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77 *Id*. at 6–7.
78 *Id*. at 7.
79 *Id*.
80 *Id*. at 9.
81 *Id*. at 12.
82 *Id*.
83 *Id*.
84 *Id.*
85 *Id.* at 21.
86 *Id.* at 22-23.

B. REPLACING, NOT REFORMING, THE 3RS

A series of articles by Ferdowsian and colleagues[87] introduces a "justice" approach to evaluating the need for animals in research. In essence, the articles take the position that the 3Rs are no longer serving the needs of society and should be replaced. Ferdowsian and Beck summarize discussions–from a workshop on implementing alternatives to animals in the biomedical sciences–by pointing out two major advancements since the publication of the 3Rs that they believe are most significant. First, a much more sophisticated understanding of animal sentience and cognition now exists.[88] According to this article, "[t]hese findings . . . challenge our assumptions about species similarities and differences and their relevance in solving ethical dilemmas regarding the use of animals in research."[89] Second, challenges to the predictive capacity of animal research have become more substantial and have reached a point where their value is small.[90] In addition, new technologically advanced methods are developing that are based on human biology and have better predictive potential.[91]

In a later paper, Ferdowsian and her colleagues more directly advocate for an anti-maleficent research agenda.[92] Continuing forward the argument about animal sentience and cognition introduced in the Ferdowsian and Beck paper, and focusing largely on the harms to animals that result from laboratory animal research, the authors propose a research paradigm that is "more ethical and just" for non-human animals.[93] Without explicitly rejecting the 3Rs, the authors' approach suggests that an entirely new ethical paradigm is needed. That justice paradigm would radically change the practice of laboratory animal research and science because a substantial amount of current laboratory animal research would no longer be acceptable. On its face, this proposal appears to bring laboratory animal use decisions into a framework that closely resembles protections afforded to humans in clinical trials.[94] In their words, "[t]he time has come for research agendas to be shaped by social, economic, and cultural values that are inclusive and morally

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87 Hope R. Ferdowsian & Nancy Beck, *Ethical and Scientific Considerations Regarding Animal Testing and Research*, 6 PLoS ONE, 1, 1 (2011); Hope R. Ferdowsian et al., *Toward an Anti-Maleficent Research Agenda*, 31 Cambridge Q. of Healthcare Ethics, 54, 54 (2022).

88 Ferdowsian & Beck, *supra* note 87, at 1–2.

89 *Id.* at 2.

90 *Id.* at 1, 2–3.

91 *Id.* at 3.

92 Ferdowsian et al., *supra* note 87, at 54.

93 *Id*. at 57; Ferdowsian & Beck, *supra* note 87, at 2–3.

94 *See generally, Regulations, Policy & Guidance,* U.S. Dep't of Health and Hum. Serv. https://www.hhs.gov/ohrp/regulations-and-policy/index.html (accessed Jan. 27, 2024) (compiling regulatory guidance and other information related to protections for human research subjects).

rigorous. Values guide science, and science cannot stand as an endeavor separate from history, social constructs, or current realities . . . ."[95]

C. GAPS IN THE 3RS

i. Training and Education of Scientists

Whether the 3Rs remain the same or evolve, the need to educate the next generation of scientists about animal use in laboratories is substantial. Russell and Burch advanced an expansive view of training and education, noting that "the educational problem here cannot be considered in isolation from the very general question of higher education itself."[96] In the two U.S. federal laws covering laboratory animal research,[97] training and education are mentioned perfunctorily and for the most part delegated to research facilities.[98]

Even after completing training requirements, many biomedical researchers are not aware of the concept of the 3Rs. [99] Franco and Olson carried out an assessment on scientists before and after their completion of a laboratory animal sciences (LAS) course.[100] They found that 58% of the respondents were not aware of the concept of the 3Rs before taking the course.[101] They point out that "a surprisingly large number of researchers were unaware of the 3Rs principle, *even those who had worked with animal models for over 10 years*."[102] After taking the LAS course, 84% of survey respondents agreed that the course positively influenced their integration of the 3Rs into their practice.[103] The insights

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95 Ferdowsian et al., *supra* note 87, at 57.

96 RUSSELL & BURCH, *supra* note 1, at 163.

97 *See supra*, Part III(A) (describing the two federal statutes, the Animal Welfare Act and the Health Research Extension Act, that address laboratory animal research).

98 *See, e.g.,* Animal Welfare Act, 7 U.S.C. § 2143(d) (1985), (stating "[e]ach research facility shall provide for the training of scientists, animal technicians, and other personnel involved with animal care and treatment in such facility as required by the Secretary." The statute further mandates that the training include instruction about (1) humane animal care and experimentation; (2) methods that limit animal pain and distress; (3) procedures that minimize or eliminate the use of animals; (4) utilization of the information service at the United States National Agricultural Library to search for alternative methods to animal use; and (5) how to report deficiencies in laboratory animal practice); *See also* Patricia Brown & Betty Goldentyer, *A Word from OLAW and USDA*, 52 LAB ANIMAL 94, 94 (2023) ("Research facilities are responsible for ensuring that personnel are qualified to perform their duties, and the regulations [9 C.F.R. § 2.32] stipulate five areas in which training must be provided . . . Regulations do not mandate a specific training frequency or format. Each research facility may determine its own training program . . . so long as the performance outcome (personnel are qualified to perform their duties) is maintained and reviewed at sufficiently regular intervals to identify deficiencies.").

99 NH Franco & IAS Olsson, *Scientists and the 3Rs: Attitudes to Animal Use in Biomedical Research and the Effect of Mandatory Training in Laboratory Animal Science*, 48 LAB'Y ANIMALS 50, 53 (2014).

100 *Id.* at 51–52.

101 *Id.* at 53.

102 *Id*. at 57 (emphasis added).

103 *Id*.

provided by this analysis highlight the necessity of implementing a more universal training plan to educate biomedical researchers in the basic concept of the 3Rs. This is both a critical present need and future goal. As this Article indicates, contemporary scientific and ethical thought about the 3Rs have advanced and become more complex. Recognizing the changes that lie ahead, a more robust training and education program will be imperative.

ii. Applicability of New Technologies and Challenges They Create

With certain revisions, the 3Rs can meet the ethical and scientific needs of toxicology, and biomedical research, for the next 65 years. They have played an important role in reducing animal pain and suffering because IACUCs must review protocols to eliminate or minimize these 'inhumanities' (in the words of Russell and Burch) that may occur during the study's lifespan.[104] Even though the 3Rs are not formally integrated into the AWA, the inclusion and acceptance of these parameters by IACUCs leads to a reduction in animal pain and suffering, in both neutral and stressful experiments.[105] While there is always room for improvement and adaptation, the 3Rs were a step forward for the animal welfare movement and provided a strong basis for the evaluation of a study's potential effects and humaneness. Overall, the implementation of the 3Rs has helped research institutions and the federal government standardize more humane practices by determining how to evaluate the appropriate number of animals needed for individual protocols, setting out procedures calling for additional review and more scrutiny when proposed protocols inflict pain that is not mitigated, and requesting that researchers utilize alternatives if animals will be subjected to pain.[106] Modern scholarship supports the notion that the 3Rs have reached a point where they are limited in advancing further reductions in animal pain and suffering; appending or innovating new approaches that do not rely on sentient beings such as animals will provide for future reduction and improvement in the humaneness of testing as more testing is required every year.[107]

The past two decades have witnessed significant changes in the development and use of new technologies, especially in the field of

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104 *See* RUSSELL & BURCH, *supra* note 1, at 14 (defining the concept of "inhumanity" in relation to the "objective assessment of the effects of any procedure on an animal subject"; 9 C.F.R. § 2.31(d) (2023) (listing the requirements for obtaining IACUC approval for animal research activities, including but not limited to: ensuring the procedures avoid or minimize animal pain, mandating the consideration of alternatives to methods causing "more than momentary or slight pain or distress," and requiring the provision of basic care for animals used in research).

105 *See Institutional Animal Care and Use Committee Guidebook*, OFFICE OF LAB'Y ANIMAL WELFARE, NAT'L INST. OF HEALTH 97 (2002) (incorporating the 3Rs in the official IACUC Guidebook's "Protocol Review Criteria"); *see generally* 30 ANIMAL LAW REVIEW (2024) Special Edition articles discussing IACUCs.

106 *See Institutional Animal Care and Use Committee Guidebook* at 86–87.

107 Ferdowsian & Beck, *supra* note 87, at 1, 3.

toxicology.[108] These technologies raise two issues that are directly related to the future of the 3Rs. First, scientific and ethical challenges raised by the 3Rs drive toward the use of technologies that do not use living beings. For example, artificial intelligence and microphysiological systems in certain situations have been used to replace some animal-based toxicology tests.[109] Obversely, these new technologies can create challenges in applying the 3Rs to their use.

Second, many biomedical researchers are currently unfamiliar with new or emerging methods of study and their use as replacements or reductions under a 3Rs scenario.[110] There has been a shift in the field of toxicology in recent years to technologies that are fundamentally in line with the 3Rs and a call for the broader scientific community to follow suit.[111] With the advent of new technologies, instruction on how to utilize and interpret these methodologies by both end-users and regulators is imperative. Some workshops and conferences have been successful in addressing this issue.[112] However, a formal framework to bridge the gap between policy and practice has yet to be established.

Few new technologies have been evaluated through a 3Rs lens. For example, as human cell based alternative techniques such as brain microphysiological[113] models become more sophisticated and can more

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108 The new technologies discussed in this section have also been employed to some extent in biomedical research and other scientific fields. For purposes of this article, the discussion is limited to toxicology because it is the author's area of expertise and education.

109 *About Alternative Methods*, U.S. FOOD & DRUG ADMIN., https://www.fda.gov/science-research/advancing-alternative-methods-fda/about-alternative-methods (accessed Jan. 24, 2024).

110 Kathrin Herrmann et al., *Food for Thought… Beyond the 3Rs: Expanding the Use of Human-Relevant Replacement Methods in Biomedical Research*, 36 ALTEX, 343, 348–349 (2019); Carl Westmoreland et al., *Use of New Approach Methodologies in Regulatory Decisions for Chemical Safety: Report from an EPAA Deep Dive Workshop*, 135 REG. TOXICOLOGY PHARMACOLOGY 105621, 105628 (2022).

111 *See generally* Herrmann et al., *supra* note 110 (encouraging the application of the 3Rs to biomedical research); *See also,* Gill Langley et al., *Lessons from Toxicology: Developing a 21st Century Paradigm for Medical Research.* 123 ENV'T HEALTH PERSP. A268, A268 (2015) (encouraging a reduced reliance on animal models).

112 Rebecca Poston et al., *Achieving Scientific and Regulatory Success in Implementing Non-Animal Approaches to Human and Veterinary Rabies Vaccine Testing*: *A NICEATM and IABS Workshop Report*, 60 BIOLOGICALS, 8, 13 (2019); Marx et al. *Biology-Inspired Microphysiological Systems to Advance Patient Benefit and Animal Welfare in Drug Development,* 37 ALTEX, 365, 390 (2020).

113 *See*, *e.g.,* Kai Wang et al., *Microphysiological Systems: Design, Fabrication, and Applications,* 6 ACS BIOMATERIAL. SCI. ENG. 3231, 3231, 3235 (2020) (noting that these systems are often derived from human stem cells or adult donor cells that have been biologically reprogrammed to develop into cell types of the tissue or organ of interest—a microphysiological system is a non-living, often engineered model of a human organ or organ function that recreates the structure and functionality of that organ (and its tissues) in a laboratory setting with enough fidelity so that, when exposed to stressors such as chemicals, it provides relevant and reliable information about how that organ will react).

accurately recapitulate human and animal brain development, a modern interpretation of ethical science must also extend to alternatives.

As emerging technologies come on the market, each will need to be evaluated for its ability to meet these three criteria. This applies to new products that can reduce the number of animals being used, which are classified as replacements. Similarly, methods that take advantage of biotechnological advances must demonstrate that they can meet the 3Rs.[114] Modern biotechnology has vastly improved our scientific understanding of toxicology and systems biology.[115] For example, 'Organ-on-a-Chip' are a new alternative that are being developed rapidly.[116] It is likely that these bioengineered devices can and will substitute for certain animal tests.[117] In other cases, non-animal methods can supplement research protocols so that the number of animals can be reduced, or a researcher might be better able to use a refinement approach to minimize or eliminate pain, stress, or distress.

V. SUMMARY AND CONCLUSIONS

The 3Rs have guided laboratory animal practice and shaped laboratory animal welfare approaches for over six decades. As scientific technologies and research questions have evolved, and as we learn more about non-human animal cognition and sentience, ethical questions about using animals in laboratories expand. As the public becomes more aware of the use of animals in research, these ethical questions will be of increasing focus for animal use in research.[118] At the same time, methods that do not use sentient beings are improving rapidly and adding robust tools for scientific exploration. Against this changing landscape, the 3Rs need to adapt and advance. As Russell and Burch point out, it remains clear that the connection between producing high quality science and treating laboratory animals ethically is a core proposition in science.[119] It is also clear that new, non-human-animal-based, technologies are developing that recapitulate human biology and have the potential to be more predictive and reliable.[120] Russell and Burch

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114 *See* Natalie Burden et al., *Pioneering Better Science through the 3Rs: An Introduction to the National Centre for the Replacement, Refinement, and Reduction of Animals in Research.* 54, J. OF AMER. ASSOC. OF LAB. ANIMAL SCI., 198, 200, 202 (2015). (calling for the use of working groups to identify areas where the 3Rs can be implemented and where additional expertise needed).

115 *See, e.g.*, Chao Ma et al., *Organs-On-A-Chip: A New Paradigm for Drug Development*. 42 TRENDS IN PHARMACOLOGY SCI., 119, 121 (Dec. 16, 2020) (discussing the contributions of organ-on-a-chip technology to biomedical research including providing biomimetic models and helping to identify drug toxicity in target organs).

116 *Id*.

117 *Id*. at 120, 130.

118 Consider, for example, the push by consumers for cruelty-free cosmetics.

119 RUSSELL & BURCH, *supra* note 1, at 4.

120 *See* Alivia Kaylor, *Alternatives to Animal Testing Models in Clinical and Biomedical Research,* XTELLIGENT HEALTHCARE MEDIA (Feb. 1, 2023), https://pharmanewsintel.com/

could not have predicted this conceptual leap in toxicological methods; one challenge that confronts society is how to calibrate this information against the 3Rs in ways that guarantee high ethical standards and advance scientific knowledge.

To maximize the utility of the 3Rs for the twenty-first century and beyond, additional principles should be added. These additions should take into consideration: (1) the need to strengthen reproducibility and predictivity of animal-based research; (2) the recognition that animals are sentient beings whose basic needs must be recognized and met; and (3) the importance of a harm-benefit evaluation that addresses the benefits, risks, and harms to humans (if certain research was not undertaken) as well as to non-human animals (who are subjected to such research). The 3Rs must evolve in these ways so that science can continue to provide value to society by advancing cures to, and treatments for, human diseases and conditions. Further, it is useful to consider how the 3Rs should be staged or valued. As new, non-animal-based, methods become more biologically relevant and are put into wider use, laboratory animal use should be reduced or replaced. An implicit concept that underlies the 3Rs is that non-animal methods should be explored before animals are used. In other words, if reduction or replacement are not feasible, then the third R—refinement—should be applied.

The wholesale substitution of the 3Rs with a justice approach is not consistent with—or necessary for—meeting today's scientific and ethical needs. The justice approach never explicitly rejects the 3Rs, but its implementation would bring laboratory animal use in close alignment to the protections afforded human populations in research settings. This change would involve a substantial ethical jump in the relationship between non-humans and humans in the laboratory setting. Among other things, research and scientific progress would almost certainly grind to a halt if a justice approach were adopted and the 3Rs were eliminated and exchanged for a system that did not balance in some way the ethical and scientific issues that laboratory animal use brings to center stage.

An open question is whether the 3Rs, or their theoretically amended principles, should be fully incorporated into U.S. federal laws that cover laboratory animal research. Legislation over the past fifty-plus years has incorporated some of the concepts underlying the 3Rs into law and regulations, but the 3Rs in their entirety enjoy recognition only because they are incorporated by reference into laboratory animal practice.

There is an urgent need for robust and effective 3Rs training. Currently, training and education are left to the research institutions, which must meet the standards outlined in the laws and regulations. As a result, training and education in the 3Rs are piecemeal, non-transparent, and highly variable. Given this background, the need for stronger training and education programs is urgent.

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features/alternatives-to-animal-testing-models-in-clinical-and-biomedicalresearch (accessed Mar. 31, 2024) (discussing the predictive capabilities of liver-on-a-chip technology and the accuracy of cell and tissue-based testing).