Non-Animal Alternatives for Research and Testing under the Animal Welfare Act: An Assessment and Vision for the Future

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1. Introduction

In 1966, the US Congress first passed legislation, which then President Lyndon Johnson signed into law, designed to address the use of some animals (largely dogs and cats) in research, testing, exhibition and transportation. The law became known as the Laboratory Animal Research Act, which was shortened to the Animal Welfare Act (AWA) in subsequent amendments. (Lee 2016; Committee on Legal Issues Pertaining to Animals 2003; Cohen 2006). In the past half century, the AWA has been amended several times, and has been extensively analyzed in both the scientific and legal literature. This article examines the relationship of the AWA to the development of non-animal alternatives and their use in scientific research, and addresses whether the AWA should be amended to enhance the development, use and future of non-animal alternatives.

Part 1 of this article consists of this brief introduction. In Part 2, the article covers the evolution of the AWA, a review of the background of regulating animal use in research, and the development of the AWA over the past half century, including an examination of its legislative history, commentaries and criticisms, with a focus on the “3Rs” (reduction, refinement and replacement – discussed herein) and non-animal alternatives. Part 3 sets out six scientific scenarios to serve as examples of the development of the science underlying alternatives, and extracts from these scenarios four principles that are important to keep in mind when evaluating potential changes in the AWA. Part 4 posits a series of questions regarding amending the AWA, with the goal of stimulating discussion about whether the AWA should or could be amended to act as a catalyst for the development and use of alternatives.

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2. The Evolution of the AWA and its policy towards animals in research

A. First steps toward today’s AWA

The issues associated with regulating the use of animals in research and science have been of long-standing concern in the United States. Efforts began in the late 19th century to control animal experimentation through legislation. For example, in 1880 a bill aimed at regulating animal experimentation in the District of Columbia (with provisions roughly similar to the 1876 British Cruelty to Animals Act) was introduced before Congress. This legislation was easily defeated because scientists opposed it. (Animal Welfare Institute, 1990 [p. 67].) Early state legislation did not address, or actively excluded, animals used in science. Between the 1820s and 1900, nearly every state and territory in the US passed anti-cruelty legislation. Fourteen of these laws explicitly exempted animals in research, and even in states without exemptions, there were no known prosecutions for animal cruelty against animal researchers before 1958. Since then, only two cases have been brought against laboratories and/or researchers based on state animal cruelty laws. (Animal Welfare Institute, 1958; Taub, 1983.) The practical impact of the Taub litigation has been to make it much more difficult to use state anti-cruelty laws as legal tools to pursue research and testing facilities and researchers. (See Reppy, W. Jr. Do State Anti-Cruelty Laws Apply to Animals Used in Scientific Research? Available at http://scholarship.law.duke.edu/cgi/viewcontent.cgi?article=2470&context=faculty_scholarship (last accessed 25 October 2016).)

At the federal level, concern about animal welfare issues was substantial by the middle of the twentieth century and led to several legislative efforts to require the humane treatment of laboratory animals. In the 1960s, the US Congress received more mail about animal care issues than about civil rights and the war in Vietnam combined. (Kreger et al. 1996, [p. vii].) Nevertheless, it was difficult to advance federal animal welfare legislation. The first animal welfare bill was introduced in 1960, and it would have required humane treatment of animals used in research by US federal grant recipients. (See 106 Cong. Rec., Part 9, pp. 1192 – 93 (1960); S. 3570, 86th Cong., 2d Sess. (1960); Animal Welfare Institute, 1990 [p. 72]) In the words of one of its sponsors, Senator William Proxmire:

“Clearly, there are two sides to the animal experimentation question. On the one hand, such experiments have made possible great scientific and medical achievements. On the other hand, some thoughtless or careless experimenters have inflicted unnecessary pain and suffering on laboratory animals. What is needed is an approach to this question which preserves the necessary and useful aspects of animal experiments while preventing the abuses.” (106 Cong. Rec. 11963).

In 1965, legislative efforts were vigorously renewed after a Dalmatian dog named Pepper was illegally taken from her owners’ home by a dog dealer. The dealer supposedly took Pepper to a hospital in New York City, but Pepper was never found. There is speculation that Pepper died on the operating table and her body was incinerated at the hospital, but it was never proven
conclusively. (Animal Welfare Institute, 1990 [p. 74]). This incident led to the introduction of HR 9743 by Congressman Joseph Resnick in the summer of 1965. This bill was amended, and later replaced by a similar bill in the House (HR 13881), and a companion bill in the Senate. After a series of hearings, amendments, and the publication of a conference report, the house bill (with amendments) was passed by both the House and Senate, and signed into law by President Lyndon Johnson in late summer 1966. (Engber 2009; CQ Almanac, “‘Dognapping’ bill enacted after heavy lobbying (1966), available at https://library.cqpress.com/cqalmanac/document.php?id=cqal66-1301593 (last accessed 20 October 2016)).

As originally enacted, the purpose of this law was:

- to protect the owners of dogs and cats from theft of such pets;
- to prevent the use or sale of stolen dogs or cats for purposes of research or experimentation; and

The 1966 legislation, defined as the “Laboratory Animal Welfare Act” in its regulations (see §1.1(a) of the 1967 rules) did not address alternatives. It did authorize the US Department of Agriculture (USDA) to promulgate regulations for humane standards, which were published in final form on 24 February 1967. (See 32 Fed. Reg. 3270 - 3282 (24 February 1967)) available at http://archive.org/stream/federalregister32aunit#page/n1422/mode/1up (last accessed 20 October 2016). These regulations cover licensing and registration of dealers and facilities and recording-keeping. They also set out certain standards for feeding, watering, sanitation and veterinary care. The standards are aimed at providing adequate husbandry, but are not directly related to elimination or minimization of pain, stress or distress. It is noteworthy that the USDA was assigned the responsibility to implement the AWA. Almost all other federal health research related legislations is under the jurisdiction of the Department of Health and Human Services (HHS).

The Laboratory Animal Welfare Act was amended in 1970 and renamed the “Animal Welfare Act.” (See PL 91-579, approved 24 December 1970.) It was also amended in 1976. While these amendments contained important changes, the changes did not directly address alternatives in research. The 1985 amendments to the Animal Welfare Act (AWA), however, included many significant changes relating to laboratory animal research and tangentially addressed alternatives.
B. The Improved Standards for Laboratory Animals Act of 1985 (ISLAA) and later amendments to the AWA

The 1985 amendments to the AWA, passed by Congress as the ISLAA, were the most significant addition to the statute from the perspective of animals used in research settings and laboratories. (Lee, 2016; Dukes 1986.) Unlike earlier amendments and the original legislation, the ISLAA authorized USDA to promulgate rules that affected animals during experimentation. Among other things, the ISLAA required:

- Minimizing pain and distress during experimentation (unless the experiment required otherwise) by requiring the use of analgesics and anesthetics;
- Establishing an Institutional Animal Care and Use Committee (IACUC) at facilities subject to the AWA;
- Training animal care personnel, including principal investigators;
- Establishing an informal service at the National Agricultural Library that would serve as a resource to reduce unintended duplication of experiments, help replace and reduce animal use, and assist in minimizing pain and distress; and
- Requiring that principal investigators consider alternatives to any procedures likely to produce pain and distress.

In addition, the USDA must inspect each facility at least one per year. (Dukes, 1986; Lee, 2016.) The ILSAA also re-affirmed a principle that was incorporated into the AWA in its 1970 amendments – the USDA was prohibited from regulating research. The research scientist still held “the keys to the laboratory door.” (Dukes, 1986 [p. 522]. More specifically, the ILSAA states that the USDA cannot regulate the design, outline, or guidelines of actual research or the conduct of actual research beyond the requirements in the law for reducing and/or eliminating pain and distress. (Cohen, 2006 [p.13]. The statutory language is found at 7 USC §§ 2143(a)(3)(E) and (a)(6)(A).)

The AWA was amended again in 1990 and 2002. In 1990, additional provisions were added to protect cats and dogs by providing a holding period before their use in research. The 2002 amendments expanded the AWA prohibition on animal fighting, and changed the AWA definition of “animal” by excluding from the definition rats, mice and birds bred for research purposes.³ (Cohen, 2006).⁴

The AWA was also amended in 2007, 2008 and 2014. These amendments strengthened animal fighting prohibitions and dog resale requirements. The 2014 amendments added a “de

³ The revised definition of “animal” is found at 7 USC §2132 (g). Section 2132 (g)(1) excludes “birds, rats of the genus Rattus, and mice of the genus Mus bred for use in research.”
⁴ Because this article is focused on alternatives it does not discuss this amendment, or its impact, in great detail. (See section 4, part iv, infra.) Obviously, the 2002 change in the definition of animal is very significant because it excludes from the AWA approximately 90 to 95% of all animals used in research. Its impact has been examined at great length in both the legal and scientific literature.
minimis” provision that allows the USDA to exempt certain small exhibitors and dealers from licensure and registration. (Cowan, 2016.)

C. Non-animal alternatives and the AWA

i. What is an “alternative?”

The understanding and evolution of the term “alternative” as applied to animals used in laboratories has a rich and complex history. (Tannenbaum and Taylor, 2015.) As a result, there is no universally agreed upon definition for this term and, especially in the legal literature, it has not been used consistently. This section of the article traces how this term has transmuted over time and offers a definition for it that is consistent with its contemporary use.

The definition of “alternatives” today is often understood to include the “3Rs” – reduction, replacement and refinement – concepts first proposed by the British scientific team of WMS Russell and RL Burch. (Russell and Burch, 1959.) The 3Rs originated as ways to diminish or remove “inhumanity” in experimentation. In their treatise “The Principles of Humane Experimental Technique,” Russell and Burch introduced these three ideas. As they explained:

“Replacement means the substitution for conscious living higher animals of insentient material. Reduction means reduction in the numbers of animals used to obtain information of given amount and precision. Refinement means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used.” (Russell and Burch, 1959 [p.66]) (Emphasis added.)

Not surprisingly, the 3Rs concepts have evolved over the past half century. The US Guide for the Care and Use of Laboratory Animals (the Guide), published by the National Academy of Sciences National Research Council (NAS 2011) and adopted by the US National Institutes of Health and AAALAC International, offer the following definitions of the 3Rs:

“Over the years, the Three Rs have become an internationally accepted approach for researchers to apply when deciding to use animals in research and in designing humane animal research studies.

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).

Refinement refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. While institutions and

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5 One of the authors of this paper (PAL) was a member of the National Academy of Sciences committee that wrote the latest version of the Guide for the Care and Use of Laboratory Animals. (NAS, 2011 p. v.)
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.

**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.” (NAS 2011 [p. __],)

The term “alternatives” does not appear in Russell and Burch’s book. It came into use later, and has been used to refer to the 3Rs collectively, as well as only one of the 3Rs -- replacement. This use of the term in both of these ways has created confusion. (Tannenbaum and Taylor, 2015.)

In this article, we adopt as the definition of “alternatives” the definition of “replacement” from the Guide. In other words, an alternative involves (1) avoiding the use of animals in experiments whenever possible (e.g., using cell culture systems or computational techniques), and/or (2) using animals that are lower on the phylogenetic scale instead of animals that are higher on the phylogenetic scale. (e.g., using a worm (c. elegans) instead of a mouse; using a zebrafish instead of a guinea pig). We use the term “non-animal alternatives” to refer to those methods and techniques that do not directly employ living creatures. In other publications, the terms “in vitro” and “in silico” are defined as examples of non-animal alternatives, and we agree with that characterization. (Lee, 2016.)

We believe that the development and use of alternatives results in stronger scientific research and the creation of better data for decision-making because the process by which alternatives are conceived, developed, tested and applied to scientific questions tends to be more forward looking and can avoid many of the scientific short-comings of traditional, animal based methods.

ii. Analysis of alternatives in the key statutory provisions of the AWA relating to laboratory animal use and research facilities that use animals

A review of the AWA’s provisions applicable to laboratory and research facilities reveals that the Act contains very few references to alternatives and no specific references to the 3Rs. A more detailed analysis of key AWA language is set out in the paragraphs that follow.

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6 As Tannenbaum and Taylor point out, in a 1995 speech WMS Russell, agreeing with one of the authors of this paper (AMG), pointed out that the use of the term “alternatives” to refer to all three Rs was both unfortunate and confusing. (Tannenbaum and Taylor, 2015 [p.123])
The AWA begins with a statement of policy. The Congressional findings do echo somewhat the concepts of replacement and reduction, although neither of these terms is used. For example, 7 USC §2131 (2) states that “methods of testing that do not use animals are being and continue to be developed ... and further opportunities exist for the development of these methods of testing,” and §2131 (3) states that “measures which eliminate or minimize the unnecessary duplication of experiments on animals can result in more productive use of Federal funds.” The plain language meaning of these statements seems to be acknowledgements that non-animal tests are evolving and that duplication is a poor use of Federal funds. These are only tangentially related to the concepts of replacement and reduction, however, and are not written as goals or objectives of the statute. The definitions set forth in the AWA 7 USC §2132 do not explicitly or implicitly identify alternatives or the 3Rs. The omission of the 3Rs from these sections is significant because if the AWA were a 3Rs law, it would be expected that in these sections replacement, reduction and refinement would at least be referenced and perhaps endorsed and/or defined.

The term “alternatives” and language that resonates with one of the 3Rs concepts is contained in 7 USC §2143, which is the central provision of the AWA applicable to research facilities. Section 2143 (a)(3) requires that the USDA promulgate minimum requirements for care, treatment and practices that ensure that pain and distress are minimized through adequate veterinary care and pain relieving drugs. It also requires that the principal investigator of an experiment consider alternatives to any procedure that would produce pain or distress. For procedures that would cause pain the AWA requires that a veterinarian is consulted, that pain killing drugs are used (unless it is scientifically necessary to withhold them, and they should be withheld only for as long as scientifically necessary); that pre- and post-operative care meets veterinary standards; and that no animal is used in more than one major operation (unless it is scientifically necessary or in other special circumstances set out by USDA). These provisions capture many of the ideas that underlie the concept of refinement, especially as that term is defined by the Guide (see above).

Section 2143 (a)(7) has requirements for research facility reporting. Facilities must report to the USDA at least annually, providing information about any painful procedures, or those likely to cause distress, and assure the USDA that the principal investigator considered alternatives to those painful procedures. Any deviations from this standard must be explained. In section 2143 (d), the AWA requires that each research facility shall train its personnel in research and testing methods that minimize or eliminate animal use, and limit pain and distress. This training is tied to the use of the National Agricultural Library, which is discussed in section 2143 (e).

iii. Does the AWA embrace alternatives and/or the 3Rs?

Based on the key provisions in the AWA that address animal experimentation and research facilities, it does not appear that the AWA directly embraces the 3Rs or alternatives in any
comprehensive or proactive way. Neither the statements of Congressional intent nor the definitions specifically mention replacement, reduction and refinement. Some of the requirements in 7 USC §2143 espouse portions of the 3Rs concepts. Perhaps the strongest evidence for assumption of one of the 3Rs – refinement – is contained in this section, which does mention the need to minimize or eliminate pain and/or distress in laboratory animals, consistent with scientific goals. Training in research methods that minimize or eliminate the use of animals is also required by the AWA. This training is linked to the establishment and use of the National Agricultural Library, which must provide information on improved methods of laboratory animal use, especially pain elimination and management and reduction and replacement. Finally, in the event that an experiment is likely to produce pain and distress, assurances must be provided that the principal investigator considered alternatives.

This conclusion that the AWA does not fully embrace or adopt the 3Rs is at odds with at least one statement in the AWA’s legislative history, public statements by the regulated community and language in several law review articles. For example, legislative history associated with the 1985 amendments to the AWA (the ISLAA) states that the amendments were intended to “reflect the importance of the 3Rs.” (Congressional Record, 1991 [p E1296].) The regulated community has acknowledged that the 3Rs are deeply ingrained in the AWA and laboratory animal practice. According to the group Speaking of Research, an advocacy organization that provides information about the importance of animal research in medical and veterinary science:

“The 3Rs are implicit in the AWA and any scientist planning to use animals (except rats, mice, and birds, which are not included in the AWA) in their research must first demonstrate why there is no alternative; and that the number of animals used, and any suffering caused, will be kept to a minimum.” See https://speakingofresearch.com/facts/animal-welfare-the-3rs/ (last accessed 6 October 2016).

One law review article accepts that the AWA is a 3Rs law:

“In 1985, the growing power of the animal rights movement, the documentation of egregious abuses of animals at several research institutions, and the general acceptance of the Three R's by advocates and researchers prompted Congress to incorporate the Three R’s into the AWA.” (Ibrahim, 2006 [p. 206]).

This belief is also adopted in a later law review article, citing as support this 2006 paper:

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7 While a detailed review of the AWA regulations is beyond the scope of this article, we believe that the regulations are consistent with the legislation in their approach to the 3Rs.

8 Interestingly, implementation of this requirement seems to be limited to a literature search for alternative procedures very late in the game. We have reached this conclusion based on a review of publicly available IACUC forms from various universities and other entities, which show that the alternatives analysis is only conducted after the experimental design has been established – and, perhaps more importantly, after funding has been awarded. For example, see http://web.jhu.edu/animalcare/forms.html (“New protocol/Third year renewal form”) last accessed 29 October 2016.
“[I]SLAA incorporated into its language a policy known at the “Three Rs” to try to increase humaneness of laboratory testing.” (Lee, 2016 [p. 200.])

Our analysis supports the conclusion that the assertion that the AWA is a 3Rs law is not correct and shows that, at best, the AWA only partially and half-heartedly captures a portion of the 3Rs concepts. We conclude that the AWA appears to be at most a “1R” law, largely aligned with the refinement principle of reducing or eliminating pain and distress when scientifically feasible.9

3. Six scenarios demonstrating the growth of, and evolution in, non-animal alternatives and four principles that emerge from them.

A. Six scenarios that demonstrate how alternatives evolve.

Before embarking on an analysis of whether the AWA should be amended to incorporate the 3Rs more fully (especially the replacement R), and (if so) how it should be amended, we believe it is important to attempt to illustrate and characterize the scope of scientific activities that are impacted by the AWA, and in particular point out similarities and differences along the continuum of scientific activities that are often lumped together as “laboratory animal science.” As explained in greater detail below, we think it is useful to parse these scientific activities into four distinct but overlapping domains, because each of these domains utilize laboratory animal science, and the data it produces, differently. To more fully describe these four domains, we first offer a series of six scenarios involving scientific problems and alternatives to animals and issues across the continuum of research. We then extract and discuss four principles that arise from these scenarios. These principles and domains will be useful in analyzing the future direction of the AWA.

ii. “The rabbit died”

In the 1950’s and 1960’s, if you heard the term “the rabbit died” it referred to someone being pregnant. Consider the popular 1960’s situation comedy, the Dick Van Dyke show. In 1962, the show ran an episode in which Mr. Van Dyke’s television wife, played by Mary Tyler Moore, announces her pregnancy with that phrase. (see http://www.imdb.com/title/tt0559862/ and http://dictionnaire.sensagent.leparisien.fr/Rabbit%20test/en-en/#cite_note-2 (last accessed 21 October 2016)). This test, developed in the 1920’s, used urine from a woman thought to be pregnant. After purification, the urine was injected into a rabbit or mouse, and after 24 hours, the animal was killed to see if it had ovulated (bloody ovaries). If yes, then pregnancy was

9 While we argue that the AWA is not a 3Rs based law, we believe that almost all animal care and use programs at institutions that use animals for research are based on the 3Rs. These programs are founded upon generally accepted practices, such as the Guide, which (along with two other standards documents) form the basis for accreditation by AAALAC International. (See http://www.aalac.org/about/guidelines.cfm (last accessed 29 October 2016)).
presumed. (Engelfried, Hawkinson and Galandey, 1945). The phrase “the rabbit died” was 1960s vernacular for a positive test.

The pregnancy had to be post 6 weeks to have adequate levels of the hormone (hCG, Human Chorionic Gonadotropin), and the test took 24 to 48 hours from injection of the urine to the killing of the animal. (Engelfried, Hawkinson and Galandey, 1945). In the 1950’s this test cost about $25 (1950 dollars) and was considered to be highly accurate.10

By the end of the 20th century, the animal test for pregnancy testing had been abandoned. It was replaced by a rapid home pregnancy test that was quick, reliable, inexpensive, and no longer required a living animal. In the privacy of one’s home, a woman can test her urine within days of a missed period (or possibly earlier) by purchasing a pregnancy test kit at the local pharmacy and within minutes have a good idea if she is pregnant. The cost of the in vitro test is usually less than $10 (2015 dollars).

In summary, the home testing kit is quicker, easier, less expensive, more reliable, and does not require the use of an animal. This is possibly the first in vitro test to receive FDA regulatory clearance and is currently the gold standard for pregnancy testing. See http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfIVD/Results.cfm (last accessed 29 October 2016).

iii. The creation of the Ames test

In the 1970’s Bruce Ames and his group published a classic paper on mutagenesis.11 (Ames 1973.) Ames’ article described a test that provides a quick, non-animal method for identifying mutagens. The Ames test uses bacteria to test whether a given chemical can cause mutations in the bacteria’s DNA. These bacteria are first modified so that they require histidine (an amino acid) for growth, but cannot produce it. The Ames test assesses the capability of a synthetic chemical that is introduced to the bacteria from a foreign source (a xenobiotic) to mutate the cells so that they can grow on a histidine-free medium. A positive test indicates that the chemical is mutagenic and, in addition, suggests that it may be carcinogenic.

As a result, the Ames test was (and still is) used to screen for possible carcinogens, although its predictive ability is relatively low, in the 50% range. The Ames test is a useful technique for screening because if a compound is found to be a mutagen using the Ames test, further testing can be used to evaluate its carcinogenicity. The test became widely used in drug development and toxicity testing, and to this day, is still considered a basic and necessary screening test for product development and safety, and required (by regulation) for registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). (Farmer, 2006).

10 Statements about the historical costs and accuracy of this test are based on personal knowledge of one of the authors (AMG), who worked as a pharmacist at the start of his career.
11 Mutagenesis is a process in which genetic information is changed, resulting in a mutation.
The Ames test was one of the first and most widely used in vitro assays. It was not developed to replace an animal test; it was created to provide more rapid, accessible and better scientific information for decision-making. However, it also became the stimulus for the development of other in vitro tests and has provided a rationale for the use of in vitro, non-animal, assays in toxicology and safety testing.

iv. The Thalidomide tragedy – overreliance on animal models

For much research, especially discovery or basic research, animal models, including rodent models, might not be a good predictor of human responses to chemicals and drugs. Rodent models are widely used and have turned out to be models of importance in many instances. There are clear examples of non-human animal studies that have contributed greatly to improving human and animal health and quality of life. (NAS, 1991.) Unfortunately, there are also numerous examples where non-human animal studies have produced misleading science, resulting in harm. Thalidomide (used as a drug to prevent morning sickness) is but one example. (See, http://www.thalidomide.ca/recognition-of-thalidomide-defects/ (last accessed 28 October 2016).) The wrong species -- dog, rat, among others -- were used to predict the human response and these studies indicated that Thalidomide was safe. We now know that the human metabolism of Thalidomide is different from animal metabolism. (Kim, 2011.) If Thalidomide is taken during a critical phase of human fetal development it can cause significant damage in humans (Brent, 1988.)

v. Advancing cell, tissue and organ cultures and organs on a chip

Cell or tissue culture is the growth of a group of cells, or tissues, independent from a living creature. Cell or tissue cultures are generally grown in a nutrient rich media (often called broth or agar). Cell and tissue cultures have many uses in scientific research, including studying how potentially toxic (or therapeutic) chemicals interact with cellular or tissue machinery. Most simple cell and tissue cultures are one dimensional or two dimensional, and are therefore limited in replicating the complex reactions of in vivo organs and living creatures. More recently, three dimensional cultures are being created. These 3D systems have greater abilities to mimic in vivo conditions and could be insightful for studying complex diseases such as cancer. (Bielecka, 2016.)

The first attempts at cell culture were carried out in the early 1800’s, and tissue culture had its start at Johns Hopkins in 1907, under the direction of Ross Harrison. (See http://www.frame.org.uk/1907-harrison-grows-from-nerve-cells-by-hanging-drop-technique/ (last accessed 28 October 2016)). Significant advances in cell, tissue and organ cultures occurred over the first 50 to 60 years of the twentieth century, and major advances continue today. Much of the work was in defining how best to grow cells and tissue in vitro. One of the first attempts to develop an in vitro mechanistic based mammalian test was undertaken by one of the authors of this paper. (Goldberg, 1980.)12

12 An abbreviated history of cell and tissue culture can be found in Appendix C of Zurlo, 1994.
Three-dimensional (3D) organ cultures have a more recent history and are becoming more common in all aspects of medical research, disease studies and toxicology (Alepee, 2014.) These technologies are advancing rapidly and have the potential to raise in vitro science to a new level of physiological relevance. At the Wyss Institute at Harvard, 3D organs are being combined with microchips and scaffolding that allows better approximation of the physical factors that affect organ function. (See https://wyss.harvard.edu/technologies/?taxonomy=focus_area&term=38 (last accessed 30 October 2016).) Using pulmonary (lung) tissue, the scaffold allows both liquid perfusion of the organ and uniquely produces stretching of the tissue mimicking respiration. Studies conducted using these technologies allow for more sophisticated research that can address complex questions and result in more relevant data. These organs on a chip are seen as the next step in advancing in vitro toxicological research. (Bahinski, 2016.)

vi. Improving toxicity testing and confronting the toxics information gap

Until its amendment this past summer, the Toxic Substances Control Act (TSCA), enacted and signed into law in 1976, was the premier US law that regulates chemicals in commerce. (See Public Law 94-469, 94th Congress, 90 Stat. 2003 (11 October 1976). In the 40 years since TSCA was passed, it has come under substantial criticism. (See, e.g., http://www.sciencemag.org/news/2015/03/congress-takes-another-crack-reforming-chemical-testing-system (last accessed 28 October 2016)). One of the major criticisms of this law was that it was not effective in producing the kind of toxicological knowledge that EPA, citizens and the business community needed to make decisions about the hazards of chemicals. (Environmental Defense Fund, 1997.) It has been estimated that of the approximately 85,000 chemicals in commerce, we have solid toxicological information on fewer than 1000. See http://thehill.com/blogs/congress-blog/energy-environment/246196-toxic-ignorance-and-the-challenge-for-congress (last accessed 28 October 2016)).

In 2007, in response to a request from the Environmental Protection Agency (EPA) and in part to address this toxic ignorance gap, the NAS published a seminal report “Toxicity Testing in the 21st Century: A Vision and a Strategy” (TT21C). The report is a detailed critique of the current animal based toxicity testing paradigm and a plan for its evolution. It recommends that the current testing paradigm should be changed in a way that allows for the development of much improved, predictive, human cell line based science.

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13 TSCA was substantially amended in June 2016, in part to try and address this toxics information gap. See Frank R. Lautenberg Chemical Safety for the 21st Century Act, Public Law 114-182, 130 Stat. 448. 22 June 2016. This change in the law does not impact the recommendations of TT21C.
TTT21C reached four major conclusions:

- Animal studies are time consuming and expensive;
- They are not always predictive of human response;
- In the future, we should encourage the use of human cells, tissues and organs for regulatory toxicity testing; and
- Systems biology and pathways of toxicity will provide better science for regulatory decision-making.

Since the report’s release, the US federal government has spent a considerable amount of time and effort to make its vision and strategy a reality. (http://www.inderscience.com/info/ingeneral/forthcoming.php?jcode=ijram. last accessed 28 October 2016.) There are now published approaches to in vitro toxicity testing for all organs of the body. Some are very advanced (e.g., skin and liver) while other are in late stages of development (e.g., the nervous system). All of these are available in academic and most industrial toxicology laboratories. The state of development is also very sophisticated in that assays can be purchased commercially, as do-it-yourself testing kits. There are now many commercial contract research laboratories, and in many cases these laboratories are devoted to in vitro studies only. (See http://www.iivs.org/ last accessed 29 October 2016.) The TTT21C report, and the new in vitro and in silico technologies, are rapidly changing the practice of regulatory toxicology. If one attends a meeting of professional toxicologists today, 80-85% of the papers presented involve the use of in vitro methodologies. In the 1970, approximately 95% of the papers at such meetings were based on animal studies. This change has been dramatic.15

B. Four principles that can be extracted from the growth and evolution of non-animal alternatives

i. There are four separate, but overlapping, domains of science that use laboratory animals.

Generally, to facilitate the discussion about alternatives, we find it useful to sub-divide the universe of animal use in science among four separate, but overlapping domains. The scientific information required in each can vary significantly. These domains are (1) discovery or basic research (e.g., developing new knowledge and tackling very complex, often cutting edge problems, such as a cure of cancer), (2) pharmaceutical and drug development (e.g., the investigation of new molecules for their potential as medicines, quest for better, more effective medicines to treat chronic conditions), (3) screening, toxicity and safety testing for chemicals used in commerce (e.g., TTT21C, TSCA and regulatory toxicology) and (4) safety and toxicity testing for cosmetics and their components.

14 This link connects to a special issue of the International Journal of Risk Assessment and Management that is devoted to the TTT21C report and its implementation.
15 Personal observation, AMG.
Discovery or basic science means experimentation and scientific exploration that seeks to find new knowledge about how the world works. More specifically, in the fields of public health and medicine discovery research is work that studies complex problems about which more needs to be known so that a cause of a disease or condition, and/or its cure, can be discerned. Discovery research is often compared to applied research, which is the utilization of science and scientific methods to design solutions, or set exposure levels or answer a legal or policy question. Basic or discovery research is also described as high risk, high pay-off research that seeks far sighted solutions. In the United States, the National Institutes of Health are one of the major funders of discovery or basic research. Academic settings (largely colleges and universities) maintain sophisticated laboratories where discovery research is conducted. In general, basic or discovery research does not seek to address “real world” problems; it is focused on creating information to fill knowledge gaps.

Science associated with pharmaceutical and drug development includes some discovery research, but also involves science aimed at practical problems. For example, in developing a potential drug molecule it is important to determine the biological mechanism by which the molecule works – that is, how it interacts with a living system – which is a basic research problem. It is also important to evaluate how a potential drug molecule can be delivered to the system of interest (i.e., lung, heart) and how much of it should be introduced (i.e., dosage), and how it should be introduced (i.e., route of administration). These scientific questions call for applied research, or research that is aimed at solving real world problems.

Screening, toxicity and safety testing for chemicals in commerce are the backbone of regulatory toxicology. Regulatory toxicology is the assessment and evaluation of information, usually by federal agencies, to determine whether exposure to a compound is safe for humans, and the levels at which such exposure is safe. In the United States, the EPA is the major practitioner of regulatory toxicology, although other agencies, such as the USDA, can play a role. Regulatory toxicology employs tests for screening compounds to make a basic determination about their safety. These tests range from very simple techniques, such as the Ames test, to more sophisticated protocols involving animals. As practiced in the United States today regulatory toxicology consumes many animals, although evolution toward non-animal methods is taking place.

Safety and toxicity testing for cosmetics is closely akin to regulatory toxicology for chemicals used in commerce. It also employs a series of tests and techniques that seek to establish whether cosmetics and their components are safe for human use, and the dosage at which they are safe. Cosmetics and their components are almost always tested for skin and eye reactions, and some of the oldest safety testing protocols, such as the Draize test, were developed for cosmetics. (Wildhelmus, 2001.) In many nations there are laws in place preventing the use of animals for cosmetics testing. For example, the European Union Cosmetics Regulation prevents the marketing of any cosmetics product if the finished product, or any component, were tested on animals after the effective dates in the Regulation. (See Regulation 1223/2009, found at
i. Non-animal methods and alternatives will be more rapidly and successfully developed if the biological mechanism(s) leading to the condition of interest are well understood and can be replicated in non-living systems.

When the biological mechanism(s) leading to the condition of interest are well characterized, it is easier to replicate them in non-living systems. This is one of the important principles to keep in mind when assessing the ease at which it is possible to move from an animal to non-animal model. The trajectory of the improvement in pregnancy testing demonstrates a relatively smooth transition from an animal to non-animal test. From its first incarnation as an animal model in the 1920s, pregnancy testing evolved relatively rapidly. The in vitro home pregnancy test of today was first developed in 1968 (patent US3579306) and found in widespread use by the late 1970’s (https://history.nih.gov/exhibits/thinblueline/timeline.html).

The so-called rabbit test is based on the biological fact that a very specific set of molecules (called, collectively, human chorionic gonadotropin, or hCG) are secreted in higher levels than normal very early in pregnancy. (Cole, 2012). These higher than usual amounts of hCG can be collected in urine and purified, and tested to show biological evidence that a woman is likely to be expecting. While the information available about hCG is much greater today than it was when the rabbit test was first proposed, the basic facts about hCG were known and understood in the 1920s. (See https://history.nih.gov/exhibits/thinblueline/timeline.html) Armed with this knowledge, two early twentieth century scientists were able to develop the rabbit test. (Ruediger, 1936.)

The greater the understanding of the biological mechanisms leading to the condition of interest, whether it is pregnancy or a health condition such as asthma, diabetes or cancer, the more likely it is to be able to effectively design experiments and carry out scientific exploration in non-living systems. A corollary to this proposition is that biological mechanisms that are more complex, and only partially understood, are usually not candidates for non-animal models. This proposition and its corollary are partially the reasons why it is feasible to eliminate the use of animal testing for many cosmetics products, which are often concerned with skin and eye irritation or corrosion (for which there is considerable scientific knowledge). By the same token, pursuit of discovery research (the study and assessment of chronic, very complex conditions, such as asthma, diabetes and cancer), are not well enough understood today to develop replacements for animal models. For studying these conditions, the default

16 A bill that is similar to this EU Regulation, called the Humane Cosmetics Act, has been introduced before the US House of Representatives. One of the authors of this paper (PAL) has publicly endorsed this bill. See https://www.scientificamerican.com/article/beauty-and-the-beasts-the-u-s-should-ban-testing-cosmetics-on-animals/ (last accessed 30 October 2016) and https://issuu.com/aavs/docs/aavs_av-magazine_2016_cruelty-free-(p.7)(last accessed 30 October 2016).

17 The original test was developed by Ascheim and Zondek. Their paper is written in German and is cited in the Ruediger article. The test first used only rabbits, but was later extended to mice and rats.
assumption in scientific research is that animal models are necessary and appropriate. Living non-human systems are considered to be accurate but imperfect replications of human systems when seeking to expand knowledge.

In contrast, for the case of toxicity or safety testing, screening tests can often be the best first step. For example, the new TSCA legislation requires prioritization (see Public Law 114-82, 114 Cong., 2d Sess., 130 Stat. 448, 22 June 2016), and alternatives can today play an important role in this process. Prioritization tests (such as the Ames test) usually evaluate a simple mechanism or reaction that will provide evidence for whether a compound should be either (1) further evaluated or (2) placed on a list of compounds that do not require additional evaluation. (Sunita, 2010.)

ii. There is a societal need and desire and increasing societal pressure for toxicity and safety testing that does not use animals.

Consumers began to demand faster and more accurate testing in the mid-twentieth century, and that contributed to the development of a faster, cheaper and predictive non-animal test for pregnancy. As we have also pointed out above, societal demand to fill the toxics information gap has contributed to a push to use in vitro toxicology to improve regulatory toxicology and safety testing. Both TT21C and the new TSCA legislation are evidence of societal response to this pressure. In addition, cosmetics companies have moved away from using animals for safety testing in part because of consumer demand and pressure from interest groups. (Basketter, 2010.)

For discovery or basic research, societal pressures to move away from animal models exist, but to a lesser extent. In the United States, attitudes toward animals in research are close to evenly split. (See http://www.pewinternet.org/2015/07/01/chapter-7-opinion-about-the-use-of-animals-in-research/ (last accessed 30 October 2016). As mentioned above, animal models are the de-facto way that biomedical research for discovery is done. Animal models are also required by regulation for developing new drugs, but alternatives are also used in this process, and with greater frequency.

Our scenarios, especially the discussions of the TT21C report and the progress of the in home pregnancy test, demonstrate that US consumers want to use fewer animals in the development of consumer products and chemicals testing. In order to meet this goal, it will be necessary to get a better understanding of human biological mechanisms.

iii. Non-animal based technologies can emerge because they represent scientific advances and are recognized as the best science.

The Ames test was created because Dr. Ames and his team were looking for a better way to test for mutagens, not because they sought to develop a replacement for an animal test. We believe that more and more in vitro tests – humane science – represent the best approaches to advancing knowledge.
In some cases, laws such as the European Cosmetic Regulation (Regulation 1223/2009, found at http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02009R1223-20150416&from=EN (last accessed 23 October 2016)) can advance the use of in vitro methods. Better science, however, is also a powerful and significant driving force. Cell, tissue and organ cultures and organs on a chip allow the use of human cells for biomedical science, the study of disease and for toxicological and safety assessment.

There are several other drivers for developing in vitro methods. As exemplified by pregnancy testing and the conclusions of the TT21C report, in vitro methods can be quicker, less expensive and, when using human cells more predictive. Animal toxicity studies are not capable of developing the data in a reasonable amount of time or at a reasonable cost. In many cases, a large shortcoming of animal studies is a lack of predictability. At a meeting at Johns Hopkins a number of years ago focusing on the dog as a model for biomedical sciences, many presentations indicated that the dog is the best model for preclinical testing of drugs for human use. As the meeting discussion developed it became clear that the dog, although the “best” model was not truly predictive for humans.

The data that was shared is that approximately 95% of drugs that were successful in the animal (dog) preclinical trials failed in humans. The FDA in the last 15 or so years requires that metabolism studies of drugs be done using in vitro human liver cultures. The failure rate of drugs so tested fell to 35%. (Albert Li, Personal communication with AMG)

As Thomas Kuhn said when describing scientific revolutions, “Each of them necessitated the community’s rejection of one time-honored scientific theory in favor of another incompatible with it. Each produced a consequent shift in the problems available for scientific scrutiny and in the standards by which the profession determined what should count as an admissible problem or as a legitimate problem-solution.” (Kuhn, 1970 [p. 6].) It is hard to predict when the scientific community will turn this corner, but there are indications that alternatives are gaining ground in all four domains.

4. Should the AWA be amended to embrace alternatives in laboratory science?

   i. Should the AWA be amended to fully adopt the 3Rs?

As a threshold question, we start by examining whether the AWA should be amended to adopt the 3Rs in full. More specifically, should the AWA be based on all three 3Rs, and in particular be more of a force in encouraging alternatives? While it is correct to say that almost all laboratory animal welfare programs are based on the 3Rs, and that the 3Rs very much inform the practical

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18 A bill called the Humane Cosmetics Act (HCA) was introduced in the US House of Representatives in 2014 that is similar in intent to the EU Cosmetics Regulation. One of the authors of this article (PAL) has co-written two articles supporting the HCA. See https://www.scientificamerican.com/article/beauty-and-the-beasts-the-u-s-should-ban-testing-cosmetics-on-animals/ (last accessed 22 October 2016 and https://issuu.com/aavs/docs/aavs_av-magazine_2016_cruelty-free- (page 7)(last accessed 22 October 2016).
decisions in laboratory animal welfare and programs, as currently written, as we pointed out earlier, the AWA is at best a 1R (refinement) law.

If the AWA were amended to incorporate the 3Rs, it would benefit laboratory animal research in several ways. First, it would make the AWA consistent with modern, humane, internationally accepted laboratory animal principles. It could be argued that for most US laboratories, this amendment would have little operational effect. These laboratories are likely certified by AAALAC International, and/or regulated by other organizations that require 3Rs compliance. As one recent law review article points out, a stronger commitment to the 3Rs could increase the recognition and use of validated alternative methods. (Lee, 2016.) Second, it might act to raise the standards of those animal research laboratories that are not applying the 3Rs, either because they are very small, or they are not required by their funding agencies to comply with the 3Rs. Third, it could be seen as a way to encourage good scientific practice. The 3Rs are affirmative duties that researchers undertake because humane treatment of animals is universally acknowledged to improve science. (NAS, 2011.)

In contrast, the full incorporation of the 3Rs into the AWA might be seen as unnecessary or ill advised. The AWA contains a clear statement that regulation is meant to stop at the laboratory door, and an AWA amendment making the Act a full 3Rs law could be interpreted as an assault on this principle, an opening of the laboratory to regulatory agencies. When properly applied, the 3Rs do seek affirmatively to probe about research question design, choice of species, number(s) of animals used, and other features of the experimental protocol. Furthermore, it could be argued that full incorporation of the 3Rs would raise costs to carry our research at institutions. (Thulin, 2014; Haywood, 2008.) Making discovery research more expensive by adding administrative burdens might be seen as a waste of governmental resources, because so much discovery work is funded by the National Institutes of Health.

ii. Should the AWA be amended to be an “alternatives first” law?

It is also important to explore whether the AWA should be amended to favor the alternatives R. In other words, if the AWA were amended so that it did incorporate all of the 3Rs, should the AWA also be amended so that it actually required principal investigators to affirmatively seek out existing, and/or develop new, experimental protocols that used non-animal methods before any laboratory animal research took place? If the AWA were amended to make it an alternatives-first law, the use of animals in science would almost certainly decrease, and animal advocates might see that as an advantage to such an amendment. Another possible advantage might be that an alternatives-first provision would spark innovation, and accelerate the development and utilization of non-animal test methods. Such a provision would fall most heavily on scientists pursuing basic or discovery research, because in this domain the default position is the creation and use of an animal model to study complex diseases and conditions. While animal models are far from perfect, and (as the Thalidomide scenario demonstrates) can miss important effects, in the opinion of most basic researchers the benefits of animal models outweigh their burdens.
For those laboratories involved in safety and/or toxicity testing, such a change would raise some, but fewer barriers. These scientists are likely using alternatives already because chemicals and cosmetics testing involves simpler endpoints and more in vitro tests are already available for use. This provision would also be consistent with the Humane Cosmetics Act, recently introduced in the US House of Representatives, which would ban animal testing on finished cosmetics products and all components. (Moran, 2014.)

Amending the AWA in this way could bring it into conflict with other federal laws, such as the Federal Food, Drug and Cosmetics Act (FFDCA), which requires certain animal tests to be performed before the FDA will consider a potential drug molecule for human clinical trials. See http://www.fda.gov/AboutFDA/Transparency/Basics/ucm194932.htm (last accessed 30 October 2016; DiSPirito,2015) and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (EPA, 2002.) However, such conflicts could be addressed by statutory drafting that excluded these regulatory testing requirements. More significantly, an alternatives-first amendment would likely increase costs associated with research and delay on-going or long standing work centered around animal models.

iii. Proposed amendments in light of the ISLAA’s Congressional findings

The ISLAA set out four Congressional findings:

“(1) the **use of animals is instrumental in certain research** and education for advancing knowledge of cures and treatment for diseases and injuries which afflict both humans and animals;

(2) **methods of testing that do not use animals** are being and continue to be developed which are faster, less expensive, and more accurate than traditional animal experiments for some purposes and **further opportunities exist for the development of these methods of testing**;

(3) measures which **eliminate or minimize the unnecessary duplication of experiments on animals** can result in more productive use of Federal funds; and

(4) measures which help **meet the public concern for laboratory animal care and treatment** are important in assuring that research will continue to progress.” (Emphasis added.)


The adoption of amendments to fully incorporate the 3Rs into the AWA would, on balance, be consistent with ISLAA’s Congressional findings. If appropriately implemented a full 3Rs strategy would recognize that certain research (especially basic/discovery research) still requires animal

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19 One of the co-authors of this paper (PAL) advocated for an alternatives-first approach to TSCA toxicity testing. (Locke, 2011)
models (finding 1). At the same time, a greater emphasis on reduction and replacement would serve finding 2, especially in the development of methods for toxicity and safety testing. Minimizing duplication would be partially accomplished by closer adherence to reduction, although reduction efforts are directly largely at keeping the number of animals in any one experiment to a minimum while maintaining the scientific value of the research. Finally, public concern for laboratory animals would be supported because the 3Rs, and a replacement-first strategy, could reduce the number of animals that are used overall.

Animal advocates have promoted both citizen suit provisions, and standing for animals, as amendments to the AWA (or other federal laws) to better police AWA enforcement and laboratory animal welfare, and as a measure to help address public concern for laboratory animal treatment (finding 4). (Frasch, 2016 (in press); Swanson, 2002.) These suggestions have been countered by pointing out that animal research is already heavily scrutinized, and the costs associated with animal research oversight represent a significant burden. (Thulin, 2014; Haywood, 2008.) This debate is likely to continue.

iv. Other possible AWA amendments

In addition to explicitly incorporating the 3Rs into the AWA as part of its policy statements, and making the AWA a replacement first law, there are other amendments that could strengthen the AWA’s commitment to the four Congressional findings set out in the ILSAA. For example, the AWA could require that facilities, through their IACUCs, keep a “3Rs scorecard.” This scorecard could track, in terms of animal use, the number of animals to which refinement was applied; the number of animals not used because reduction measures were put into place; and the number of animals replaced by non-animal alternatives. In addition, the AWA could require that these reports are made available to members of the public.

The IACUC provisions of the AWA could also be changed. The ISLAA amendments to the AWA established these internal committees at facilities to oversee animal use in research. The AWA requires the appointment of an individual to the IACUC who can represent societal interests. This provision could be made more effective in bringing the public’s voice into research facilities and laboratory animal welfare decision-making. In a system of self-regulation and oversight such as the one established pursuant to the AWA, it is imperative that this public representative be effective and informed.

Training is another area where improvements could be made. As it is currently written and implemented, the training required under the AWA is left to each facility or institution. Perhaps training should be standardized, and the AWA should be amended to establish a national educational body whose mission is to train laboratory animal researchers. Each researcher and laboratory worker would be required to take a standardized curriculum, and that curriculum could be focused on the four Congressional findings in the ISLAA.

Finally, it is important to consider an amendment to the AWA that would change the definition of “animal” so that it does not exclude rats, mice and birds bred for research purposes. For the
protections and benefits to laboratory animal welfare and scientific research to be fully realized, it would seem obvious that the bulk of animals used in research should not be excluded by this definition from the AWA. Changing the definition of animal in this way would make the AWA more consistent with other federal requirements that govern laboratory animal research (See http://grants.nih.gov/grants/olaw/references/phspolicylabanimals.pdf (last accessed 30 October 2016), setting out the requirements for animal research for recipients of funds from the Public Health Service, including a definition of “animal” that does not exclude rats, mice and birds bred for research purposes; see also the Guide, which defines an animal as “as any vertebrate animal (i.e., traditional laboratory animals, agricultural animals, wildlife, and aquatic species) produced for or used in research, testing, or teaching.”)
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