

# Animals in Research: Contemplating the Need

Chetan Bakshi and Veena Dhawan

Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

**Corresponding author:** Veena Dhawan, Professor, Department of Experimental Medicine and Biotechnology, Research Block-B, Postgraduate Institute of Medical Education and Research, Chandigarh, India, E-mail: [veena447@gmail.com](mailto:veena447@gmail.com)

## Abstract

Undoubtedly, animal experimentation has played a central role in biomedical research throughout history, where animals were first used as models of anatomy and physiology. At present, animals in research are essential not only in areas of neurological, infectious, digestive, genetic and chronic diseases, but also play a crucial role in the development of antibiotics, vaccines as well as in proper understanding of various diagnostics and prognostic tests.

Animals such as rats, mice, rabbits, guinea pigs, frogs, fish, birds, sheep, pigs, dogs and primates, provide very useful models in research. There are seven major areas of medicine and biology, where animals are used, but majority of animal models are used to study the cause, nature and cure of diseases affecting both humans and animals. Since, new animal models are continually being characterized, identifying and selecting the most appropriate animal model is the single most essential element in animal-based research. When the use of laboratory animal is obligatory, the most appropriate species, breed, and strain with the closest homology to humans must be chosen. Additionally, improved reporting of the details maximizes the availability and utility of the information gained from every animal experiment, also preventing the unnecessary animal use in the future.

It is upon the researcher to ensure that research involving animals will contribute significantly to the health and welfare of either humans or animals. It is our moral duty to ascertain that animal experimentation should cause as little suffering to animals, as possible and should only be performed where necessary.

**Keywords:** Animal research; Experimental animals; Animal model; Animal welfare

## Background

Humans have been using animals as models of their anatomy and physiology since the dawn of medicine. Ancient Greek physicians dissected animals for anatomical studies because of the taboos regarding the dissection of humans<sup>[1]</sup>. Prominent physicians from this period who performed “vivisections” (stricto sensu the exploratory surgery of live animals, and historically used lato sensu as a depreciative way of referring to animal experiments) include Alcmaeon of Croton (6<sup>th</sup>–5<sup>th</sup> century BC)<sup>[2,3]</sup>, Aristotle, Diocles, Praxagoras (4<sup>th</sup> century BC), Erasistratus, and Herophilus (4<sup>th</sup>–3<sup>rd</sup> century BC)<sup>[1,2,4]</sup>. Modern research principles can be attributed to three physiologists from the 1860s. In 1865, Claude Bernard, a French physiologist published a book, *An Introduction to the Study of Experimental Medicine*, intended to provide guidance to physicians in experimental research<sup>[5]</sup>. It became the first published book to advocate creating “induced animal models” for biomedical research, by proposing the use of chemical and physical induction of disease in animals. His peers of the time, Louis Pasteur and Robert Koch introduced the concept of specificity into medicine and the “germ theory of disease.” The turning of the century saw the development and

use of animal models for infectious diseases and screening and the evaluation of new antibacterial drugs based upon the work of these three researchers.

Human biology is very much similar to that of many other animals. That is why; results from animal experimentations are applied to humans. Most laboratory animals have the same set of organs - heart, lungs, liver, and so on which work in the same way as they do in human. There are seven major areas of medicine and biology, where animals for experiments are used:

**Received date:** March 23, 2018

**Accepted date:** February 28, 2019

**Published date:** March 5, 2019

**Citation:** Dhawan,V. Animals in Research: Contemplating the Need (2019) J Vet Sci Ani Wel 3(1): 5-12.

**Copy Rights:** © 2019 Dhawan,V. This is an Open access article distributed under the terms of Creative Commons Attribution 4.0 International License.

**Fundamental biological and medical research:** If we essentially know how different tissues and organs are kept healthy, we can then find out what goes wrong during disease. In the past, animal research played a vital role in discoveries such as how the kidneys work, or how hormones control different parts of the body. At present, basic research in many areas of biology and medicine still needs to use animals. A good example would be the brain, since there is still a lot we do not know about how it works and if we are to find answers to these important questions, fundamental research must continue. The information acquired by fundamental research often provides new insights into more applied medical research that ultimately leads to the development of new medicines.

**Developing treatment for diseases:** To overcome a disease, a lot of work needs to be put into, by way of developing better medicines, perfecting surgical operations as well as making vaccines and finding other ways of preventing diseases. Examples include development of polio and diphtheria vaccines, insulin for diabetes, and kidney transplantation which depended on animal research. There are many diseases which are yet to have a proper cure like multiple sclerosis and certain cancers, as well as diseases like AIDS, Alzheimer etc. All these need initial input, especially in terms of animal experimentation.

**Preparations of products used in medical research and treatment:** Animals can produce useful medical substances in their blood or milk, like antibodies, vaccines and hormones, which are important for diagnostic tests, medical treatments and basic research. For example, a vaccine against tetanus is tested for potency in mice or guinea pigs.

**Safety testing of chemicals and drugs:** A wide range of chemicals and medicines which are used in day-to-day life, need to be tested for their safe use in humans as well as in animals. New drugs are often required to be tested in at least two animal species in preclinical trials before moving on to human clinical trials<sup>[6]</sup>. Many of these tests are exceptionally inhumane and involve high levels of pain and distress for a range of species from rodents to non-human primates (including chimpanzees).

**Study of genetic disorders:** There are many diseases which are inherited fully or partially from parents to their offspring and are caused by basic aberrations in a person's genetic code. Some of the animals also have similar genetic faults as humans do. There are mutant strains like dystrophic mice, which have the same faulty gene as found in the muscular dystrophy patients. The majority of genetic studies, have employed mice, not only because their genome is similar to that of humans, but also because of their availability, ease of handling, high reproductive rates, and relatively low cost of use. Other common experimental organisms include fruit flies, zebra fish, and baker's yeast. Scientists have now made such advancements in molecular biology that they can now alter genes and even breed strains of mice and other animals with particular genetic diseases. This may ultimately show the way to find treatments of genetic disorders like cystic fibrosis, sickle cell anemia and other diseases which run in families. Mouse models have helped in understanding the role of chloride channels in cystic fibrosis, a major killer of young adults.

**Development of new diagnostic tests for diseases:** For the treatment of a disease is to be effective, a quick and precise diagnosis is essential. Animal experiments play a vital role in this area, which include developing techniques such as scanning to check on the health of unborn babies, to identify some cancers, to diagnose heart disease. Studies in sheep and lambs led to the use of steroids in the treatment of respiratory distress syndrome (formerly hyaline membrane disease), a major cause of death in premature infants. Apparently, animal experiments have paved the way for the development of many important blood and tissue markers of infection<sup>[7]</sup>.

**In biology and medical education:** The animals are also used for teaching biology in schools, colleges and universities, to provide better understanding of the basic anatomy and physiology of man and other animals. Frogs, fetal pigs, perch, cats, earthworms, grasshoppers, crayfish and starfish are commonly used in classroom dissections<sup>[8]</sup>. On the contrary, there are efforts in many countries to find alternatives to use animals in education. The NORINA database, maintained by Norecopa<sup>[9]</sup>, lists products that may be used as alternatives or supplements to animal use in education, and in the training of personnel who work with animals<sup>[10]</sup>. Inter NICHE has a similar database and a loans system.

Animals are frequently used in many areas of biomedical research, such as rabbits are used in toxicity and safety testing of medical devices, vaccines, and drugs, whereas guinea pigs and hamsters, are used in toxicity testing and as models for infectious, cardiovascular, and neurological diseases, and drug abuse research. Mice and rats are commonly used in vaccine and drug research and efficacy testing, while birds are used in research on organ development and deformity, muscular dystrophy, visual impairment, and nutrition. Research generally involves three facets: gaining new knowledge, use of animals in educational institutions for teaching and for the testing of new drugs, chemicals or devices for their safety and effectiveness. In recent times, numerous aspects of normal and disease biology, including the biology of small regulatory RNAs (e.g., RNAi, microRNAs, snoRNAs), the importance of copy number variation and non-coding regions of DNA in the determination of disease phenotypes, the importance of epigenetic regulation, and the biology of stem cells and its implications for normal and aberrant cell differentiation, have come to light<sup>[11]</sup>. In addition, more specific areas of disease biology such as the importance of epithelial to mesenchymal transition in cancer biology, mechanisms of genomic instability that can lead to cancer, and the role of cilia in normal development and in various diseases have also emerged recently<sup>[11]</sup>. Similarly, new drug research as well as tests meant for assuring the quality and efficacy of pharmaceutical products /vaccines / any other biological is absolutely based on experiments involving animals. No new drug can be introduced in clinical practice or even for the matters into clinical research unless it has passed an array of toxicity tests in animals. Studies involving animal models, including the development of new disease models, have assisted humans in gaining knowledge about these areas of normal and disease biology, and will continue to do so in future.

### Classification of animal models

According to the U.S. National Research Committee on Animal Models for Research on Aging the term “laboratory animal model” implies as “an animal in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more aspects resemble the same phenomenon in humans or other species of animal.”

An animal model is considered as homologous if the symptoms shown by the animal and the course of the condition are similar to those of humans. For instance, the discoveries of swine hepatitis E virus (HEV) from pigs and avian HEV from chickens provided an opportunity to develop small homologous animal models for HEV<sup>[12]</sup>. An animal model is considered isomorphic if the animal’s symptoms or anatomy is similar, but the etiology or genetic character is different. For example, the human and mouse heart shows a set isomorphism at the organ level and also at the organ part level. However, most of the animal models may be termed as partial, since they are neither homologous nor isomorphic. These models generally do not mimic the entire human or animal disease, but may be used to study certain aspects or treatments of the disease. For instance, animal models of Alzheimer’s disease can be created based on the accumulation of amyloid- $\beta$  peptide in the brain and have many amyloid plaque deposits; however, they have only subtle behavioral and electrophysiological deficits, thus provide only a partial model of the human condition<sup>[13]</sup>.

When animals are used in research to study biological and functional systems in humans, they are divided into the following categories:

**Exploratory:** When animals are used to understand the fundamental biological mechanisms, whether it is normal or abnormal. For instance, a novel animal model used for the study of aging, particularly for identifying genes and biochemical pathways regulating longevity. The laboratory mice and rats are the commonly used models for the study of aging and age-related diseases. Indeed, rodents paved the way for both dietary and genetic interventions in aging, as best illustrated by the discovery that calorie restriction extends the rodent life span, as well as the finding that mutations in certain genes are associated with longevity. Additionally, rhesus macaques (*Macaca mulatta*), roundworms (*Caenorhabditis elegans*) and fruit fly (*Drosophila melanogaster*) are among commonly used models in aging research.

**Explanatory:** When animals are used to gain an understanding of multifaceted or complex biological problems. An example would be the use of cognitive and psychosocial animal models to provide etiology for anorexia nervosa<sup>[14]</sup>. Rats and mice are among the commonly used stress, activity and diet restriction models of anorexia nervosa. The mouse with autosomal recessive *anx* mutation and neuropeptide Y (NPY) knockout mouse represent genetic models of anorexia.

**Predictive:** When animals are used to investigate the efficacy of investigative treatments, whether for diseases or chemical toxicities. Predictive animal testing models are important in improving the success of a drug or medical device in clinical trials and

for generating new data in support of existing products. The first antipsychotics (chlorpromazine and haloperidol) served as standards for characterization of the early animal models. Generally, these models consist of certain responses exhibited by rodents (mice and rats) after pharmacological, genetic or developmental interventions<sup>[15,16]</sup>. Finally, some tests including assessment of social interaction, the object recognition test, and the Morris water maze can predict drug effects on negative and cognitive symptoms of an animal model.

The majority of laboratory animal models are developed and used to study the cause, nature and cure of human diseases. Disease models may suitably be categorized in one of the following five groups, of which the first three are most important:

**Induced (experimental) models:** Normal animals are experimentally induced to create these models by means of invasive procedures, such as surgeries, traumatic injuries, burns, force-feeding, blood withdrawals or biopsies, food or water or social deprivation, behavioral or environmental manipulations, dart gun sedation, prolonged restraint, viral or bacterial infections and exposure to toxic drugs and chemicals. For example, creating heart attacks, heart failure, abnormal heart rhythms, strokes, and other cardiovascular traumas in monkeys, dogs, pigs, and other animals; inducing symptoms of migraines in cats and primates through brain stimulation and manipulation with chemicals; implanting electrodes into the intestines of dogs to induce motion sickness and vomiting; implanting electrodes into the brains and eyes of monkeys and cats to conduct neurological and vision experiments; and dropping weights onto rodents to produce spinal cord injuries and paralysis<sup>[17]</sup>.

**Spontaneous (genetic) models:** Spontaneous models mimic the human condition due to genetic variance, which occurs naturally through mutation and not due to experimental induction. The spontaneous mutation of *nu* among laboratory mice (produces a hairless state, named as “nude”) led to the nude mouse becoming the first animal model of a severe immunodeficiency. The unique defect of nude mice is the failure of the thymus to develop normally to maturity, thus produces reduced numbers of mature T cells. This means nude homozygotes (animals with identical mutant genes at corresponding chromosome loci) do not reject allografts and often do not reject xenografts (tissue from another species). Since then, the nude mouse is commonly used model for studying the factors regulating transplantable human tumor growth and cancer metastasis<sup>[18]</sup>. Additionally, it has been noted that certain strains of inbred mice are pre-disposed to develop cancer (skin, breast and leukemia).

**Transgenic models:** Transgenic animal models are the induced models in which DNA is either inserted into or deleted (knock-out) from the genome of the animal. The term “transgenic” was coined in 1981 by Gordon and Ruddle to describe an animal in which an exogenous gene was introduced into its genome. In the late 1980s, the utility of transgenic technology was extended to gene-targeting experimentation as well as for the production of chimeric or “knockout” mice in which a gene (or genes) has been selectively removed from the host genome<sup>[19]</sup>. Recently, our ability to manipulate the mouse genome has become increas-

ingly refined with developments such as tissue-specific methods of knocking out genes such as the Cre-Lox system, methods of turning on or off gene transcription *in vivo* using tetracycline- or tamoxifen-induced systems, and methods of identifying or removing entire cell lineages *in vivo* via fluorescent protein- and diphtheria-toxin receptor-knock-in mice respectively. Additionally, using similar technology transgenic rats, cats, dogs, rabbits, pigs, sheep, goats, cattle, chickens, zebrafish, and non-human primates, have also been generated. Nowadays, humanized models such as transgenic animals expressing human genes are also being employed. A classic example involves the insertion of the gene encoding the human major histocompatibility locus, HLA-B27 into rats. Individuals with this MHC haplotype have increased susceptibility to several autoimmune conditions.

**Negative models:** Negative animal models fail to respond to a disease or a chemical stimulus. Thus, they are most commonly used to study the mechanism of disease resistance. A classic example is the failure of gonococcal infection to develop in rabbits after an experimental treatment that induces the disease in other animals. These models have become increasingly important with the advent of transgenic technology. For example, a novel transgenic mouse was created to study the lack of development of autoimmune thyroiditis with the injection of self-thyroglobulin lacking certain surface epitopes to account for the negative reaction<sup>[20]</sup>.

**Orphan models:** Orphan animal models are the opposite of negative animal models, since disease occurs in animals, but there is not a corresponding disease in humans. However, Orphan models may become induced models when a similar disease is recognized in humans later on. Historically, scrapie in sheep was such a model, but now is useful as a model for the human spongiform encephalopathies that are of so much concern (e.g., BSE, “mad cow disease,” and CWD, chronic wasting disease in deer).

#### Animal model of choice

Laboratory animal refers to any species of animal undergoing an experimental procedure in a research laboratory or formal test setting. Hence, identifying and selecting the most appropriate animal model is the single most essential element in animal-based research and also challenging at the same time. Philosopher, Bernard Rollin quoted that “The most brilliant design, the most elegant procedures, the purest reagents, along with investigator talent, public money, and animal life are all wasted if the choice of animal is incorrect.” Worldwide, before animal research is approved, most animal welfare legislation requires consideration of alternatives. Checking literature sources and conducting searches in bibliographic databases are the common methods to insure compliance<sup>[21]</sup>.

Since, new animal models are continually being identified and characterized, the most appropriate species, breed, and strain with the closest homology to humans must be chosen, when the use of a laboratory animal is obligatory. The search for the appropriate animal model should involve a thorough literature search and a check of appropriate websites. These web-based resources include AltWeb, in particular the Pain and Humane Endpoints databases<sup>[22]</sup>; ANZCCART, in particular the Fact Sheet on Pain<sup>[23]</sup>; Ensembl Genome Browser<sup>[24]</sup>; International

Mouse Strain Resource, IMSR<sup>[25]</sup>; Mouse Models of Human Cancers Consortium<sup>[26]</sup>; Isogenic Info<sup>[27]</sup>; and, the UKCCCR Guidelines<sup>[28]</sup>. In addition, Animal Welfare Information Center (AWIC) offers profound information, including the “Alternatives” section<sup>[29]</sup>. It provides information and links to techniques, methods, procedures, and animal models. The Institute of Laboratory Animal Research (ILAR) also maintains a very practical and useful database on animal models and strains<sup>[30]</sup>. Selection of a species should not be based solely on availability, familiarity, or cost. Animals fulfilling these criteria may not provide the genetic, physiological, or psychological aspects required for the proposed question. It is almost impossible to adhere to specific rules for the choice of the best animal model, because of many considerations that have to be made before an experiment can take place differ with each research project and its objectives. Nevertheless, some general rules can be considered which include research factors, animal-care factors, physical and environmental factors.

#### Description of animal models

Failure to describe research methods and to report results appropriately involving animals have a potential scientific, ethical, and economic implications for the entire research process and the reputation of those involved in it. For instance, according to the survey, commissioned by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), a UK Government-sponsored scientific organization, found that only 59% of the 271 randomly chosen and assessed articles stated the hypothesis or objective of the study, and the number and characteristics of the animals used (i.e., species/strain, sex, and andage/weight)<sup>[31]</sup>. Ideally, scientific publications should present sufficient information to allow a knowledgeable reader to understand what was done, why, and how, and to assess the biological relevance of the study and the reliability and validity of the findings. There should also be enough information to allow the experiment to be repeated<sup>[32]</sup>.

Improved reporting of the details may maximize the availability and utility of the information gained from every animal and every experiment, also preventing the unnecessary animal use in the future. To address this issue, the guidelines for reporting research involving animal experimentation have been developed using the CONSORT Statement as their foundation and are referred to as ARRIVE (Animals in Research: Reporting *In Vivo* Experiments)<sup>[33,34]</sup>. The ARRIVE guidelines consist of a checklist of 20 items, which entitles all scientific publication reports using animals to include the minimum information including the number and specific characteristics of animals used for the purpose (species, strain, sex, and genetic background); details of animal housing and husbandry; and the experimental, statistical, and analytical methods used (including details of the methods used to reduce bias such as randomization and blinding). All the items in the checklist have been included to promote high-quality, comprehensive reporting to allow an accurate, critical review of what was done and what was found<sup>[33,34]</sup>. Unlike the old days when the researcher could write in the materials and methods section “white mice were used in the study”, modern obligations require a precise scientific description of the model animal used.



**Invertebrates:** Several invertebrate systems are considered as suitable alternatives to vertebrates in early-stage discovery screening. The most frequently used invertebrate species are *Drosophila melanogaster*, a fruit fly, and *Caenorhabditis elegans*, a nematode worm. These invertebrates offer some advantages over vertebrates in animal testing, including their short life cycle and the ease with which large numbers may be housed and studied. Because of similarities between the innate immune system of insects and mammals, insects can replace mammals in some types of studies. However, the lack of an adaptive immune system and their simple organs, prevent worms from being used in several aspects of medical research such as vaccine development. Similarly, *Drosophila melanogaster* immune system differs greatly from that of humans, and diseases in insects can be different from diseases in vertebrates; however, fruit flies and *Galleria mellonella* waxworm can be useful in studies to identify novel virulence factors or pharmacologically active compounds. Adopting such models generally involve accepting a lower degree of biological similarity with mammals for significant gains in experimental throughput.

**Vertebrates:** Mice are the most commonly used vertebrate species in biomedical research because of their small size, faster reproduction, ease of handling and low maintenance cost. Since, mice share 99% of their genes with humans, they are widely considered to be the best model of inherited human disease. With the advent of genetic engineering technology, genetically modified mice can be generated and can provide models for a broad range of human diseases. Another vertebrate species widely used for physiology, toxicology and cancer research are rats, but genetic manipulation is more difficult in rats than in mice, which limits the use of these rodents in basic science. Other commonly used rodents are guinea pigs, hamsters, and gerbils. Fish and amphibians are also commonly used vertebrate species. The main species used are the zebrafish, *Danio rerio*, which are translucent during their embryonic stage, and the African clawed frog, *Xenopus laevis*.

Albino rabbits are used in eye irritancy tests (Draize test) because rabbits have less tear flow than other animals, and the lack of eye pigment in albinos make the effects easier to visualize. Rabbits are also frequently used for the production of polyclonal antibodies. Cats are most commonly used species in neurological research. Dogs are widely used in biomedical research, testing, and education particularly beagles, because they are smaller in size, gentle and easy to handle due to an amenable temperament. They are used as models for human diseases in cardiology, endocrinology, and bone and joint studies, research that tends to be highly invasive, according to the Humane Society of the United States.

**Nonhuman primates:** Nonhuman primates (NHPs) are predominantly used in toxicology tests, studies related to neurology, behaviour and cognition, reproduction, genetics, and xeno-transplantation. Because of the genotypic and phenotypic resemblance to humans, nonhuman primates have been used in the study of induced or naturally occurring human diseases such as acquired immunodeficiency syndrome (AIDS), hepatitis, diabetes mellitus, and atherosclerosis. They are caught in the wild or purpose-bred or ethically obtained from large feral

populations. In the United States and China, most primates are domestically purpose-bred, whereas in Europe the majority are imported purpose-bred. NHP is generalized as one animal in literature, whereas NHPs encompass a variety of species ranging from lower forms such as the tree shrews as small as several grams to the great apes as large as more than 100 kg. Most of the NHPs used in experiments are macaques, but marmosets, spider monkeys, and squirrel monkeys are also used, and baboons and chimpanzees are used in the US. The first transgenic primate was produced in 2001, with the development of a method that could introduce new genes into a rhesus macaque. This transgenic technology is now being applied in the search for a treatment for the genetic disorder Huntington's disease. Notable studies on non-human primates have been part of the polio vaccine development, and development of Deep Brain Stimulation, and their current heaviest non-toxicological use occurs in the monkey AIDS model, SIV. In 2008 a proposal to ban all primate experiments in the EU has sparked a vigorous debate. Nevertheless, the humane use of nonhuman primates as a physiological, pharmacological, and toxicological research model is critical for safety assessment of new drugs and biotechnology products.

Many establishments conduct biomedical research which includes universities, medical schools, defense establishments, pharmaceutical companies, farms and commercial facilities that provide animal-testing services to industry. Sources of laboratory animals vary between countries and species. An animal source is governed by directives which require animals to be specially bred for research purposes, unless the animal has been lawfully imported and is not a wild animal or stray. Sources include commercial breeders, businessmen, dealers, animal shelters etc. Large centres are also there which distribute strains of genetically modified animals such as transgenic, knock-in and knock-out animals.

In spite of the advancement in biomedical research, and the benefits derived by the society through them, the opposition to animal experimentation always existed. It has a long history, dating almost from the day animals were being used in biomedical research. Due to deep concerns regarding animal welfare, formulation of the Institutional Animal Ethics Committee is a mandatory step in institutions conducting animal research. Their objective of utmost importance is to ascertain rational and humane use of animals in research. At the same time, it ensures prevention of unnecessary pain or suffering or injury to animals during holding, experimentation and post-experimentation period by monitoring and improving their housing, environment, feeding and veterinary care. This is achieved by providing accreditation services to laboratories by constituting, National Accreditation Board of Testing and Calibration Laboratories (NABL) having membership of the International Laboratory Accreditation Cooperation. Such accreditation of animal facilities demonstrate their commitment to responsible animal care and use and good science since such an accreditation is an indicator of an institution's ability to comply with its assurances. At present, many rules and acts are enacted at the international level, to protect the animals against the cruelty and misuse. Many organizations provide the guidelines for animal house keeping, breeding, feeding, transportation and mainly for their ethical use in scientific experiments. Such organisations include ICH (International Conference on Harmonization of technical

requirements for registration of pharmaceuticals for human use), CPCSEA (Committee for Purpose of Control and Supervision on Experiments on Animal), NIH (National Institute of Health) and OECD (Organization for Economic Cooperation and Development)<sup>[35]</sup>.

In recent years, there has been an increasing tendency for restricting the use of laboratory animals in studies of new drug development and at the same time with no compromise for their safety in humans<sup>[36]</sup>. A series of European directives and guidelines recommend limiting the use of experimental animals on the grounds of ethical and scientific rules and regulations for animal protection. The three R's proposed by Russel and Birch<sup>[37]</sup> (1959) are the guiding principle as an alternative for the use of animal research:

- Reduction – refers to obtain comparable levels of information by using less number of animals, avoiding replication of studies or avoiding studies in animals which are not relevant or improving the quality of experiments.
- Refinement – refers to methods reflecting the scientific and technical progress which alternate or minimize potential pain, suffering or distress, and enhance the quality of life of animals during the experiments.
- Replacement – refers to preferred use of alternative to animal models, such as *in vivo* and *in vitro* techniques based on human cells to possibly achieve the same scientific aim.

The importance of their approach lies in its combination of animal welfare considerations along with good science and best practices. When discussing the 3 Rs, invariably Replacement is the R most often considered. While primary and utmost effort should be given to developing and using *in vitro* alternatives, the other 2 Rs – Reduction and Refinement – are essential considerations in research as well and are too often overlooked. Replacement is the ultimate alternative in animal welfare measures, where given purpose is achieved without conducting experiments or other scientific procedures on protected live animals<sup>[38]</sup>.

Considering the possibility of extinction, the European Union, in November 2008, put forward the proposals to ban the use of great apes, chimpanzees, gorillas and orangutans in scientific procedures other than in exceptional circumstances. The ethical questions raised by performing experiments on animals are subject to, lots of criticism and controversies and a debate, ensues for and against whether animal should or should not be used for experimental research. In 1860, Henry Bergh founded the American Society for the Prevention of Cruelty to Animals (ASPCA) and also the Laboratory Animal Welfare Act was passed in 1966. It is mandatory for the researchers to take prior permission of the Institute's Animal Ethics Committee before any research based on animals is funded. There are strict regulations regarding animal euthanasia. Strict regulations govern animal experimentation and under provisions of the Act and the Guide for the Care and Use of Laboratory Animals (the Guide), published by NAMS, any procedure can be performed on an animal if it is proven that it is scientifically justified.

### Concluding remarks

Laboratory animals occupy an essential position in biomedical research, drug discovery as well as technological advances and they will continue to improve the lives of people and other

animals. Nowadays, researchers rely on the identification and development of animal models to explore all avenues of medical science to include assessment of pathogenic mechanisms, diagnostic and therapeutic procedures, nutrition and metabolic diseases, and the efficacy of novel drug development. It is incumbent upon the researcher to know the subject well in order to provide relevant information to the scientific world. The final judgment in the choice of the animal model will always be in its ability to elucidate and predict the observed effects in the target species. There must be reasonable expectation that research involving animals will contribute significantly to present and future knowledge, which may eventually lead to the protection and improvement of the health and welfare of either humans or animals. Thus, it becomes our moral duty to see that animal testing should cause as little suffering to animals, as possible and should only be performed where necessary.

### Declarations

Ethics approval and consent to participate  
“Not applicable”

### Consent for publication

“Not applicable”

### Availability of data and materials

“Not applicable”

### Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

### Authors' contributions

The authors contributed equally in writing the manuscript. All authors read and approved the final manuscript.

### Acknowledgements

“Not applicable”

### References

1. Staden, V.H. Herophilus: The Art of Medicine in Early Alexandria: Edition, Translation and Essays. Cambridge University Press: Cambridge; 1989.  
Pubmed| Crossref | [Others](#)
2. Maehle, A.H., Tröhler, U. Animal experimentation from antiquity to the end of the eighteenth century: Attitudes and arguments. In: Vivisection in Historical Perspective. Rupke NA, editor. Croom Helm: London; 1987.  
Pubmed| Crossref | [Others](#)
3. Court, W.E. Pharmacy from the ancient world to 1100 AD. In: Making Medicines: A Brief History of Pharmacy and Pharmaceuticals. Anderson S, editor. Pharmaceutical Press: London; (2005).  
Pubmed| Crossref | [Others](#)

4. Geller, M.J., Phlegm and breath—Babylonian contributions to Hippocratic medicine. In: *Disease in Babylonia*. Finkel IL, Geller MJ, editors. Brill: Netherlands. (2007).  
[Pubmed](#) | [Crossref](#) | [Others](#)
5. Bernard, C. *Introduction to the Study of Experimental Medicine*. Dover Publications Mineola, New York, USA, 1957 (originally published in 1865; first English translation by Henry Copley Greene, published by Macmillan & Co., Ltd., 1927).  
[Pubmed](#) | [Crossref](#) | [Others](#)
6. Brewer, T. Trials and Errors: Drug testing raises ethical – and efficacy – issues. *Best Friends Magazine*, 2007, Best Friends Animal Society, Kanab, Utah.  
[Pubmed](#) | [Crossref](#) | [Others](#)
7. Griffin, J.F. A strategic approach to vaccine development: animal models, monitoring vaccine efficacy, formulation and delivery. (2002) *Adv Drug Del Rev* 54(6): 851–861.  
[Pubmed](#) | [Crossref](#) | [Others](#)
8. Orlans, F.B., Beauchamp, T.L., Dresser, R., et al. *The Human Use of Animals: Case Studies in Ethical Choice*. Oxford University Press, England, (1998).  
[Pubmed](#) | [Crossref](#) | [Others](#)
9. The NORINA database of alternatives. *Oslovet.norecopa.no*.  
[Pubmed](#) | [Crossref](#) | [Others](#)
10. Dalal, R., Even, M., Sandusky, C., et al. Replacement Alternatives in Education: Animal-Free Teaching (Abstracts to the Fifth World Congress on Alternatives and Animal Use in the Life Sciences, Berlin). *ALTEX*. 2005; 22:5-348.  
[Pubmed](#) | [Crossref](#) | [Others](#)
11. Haberman, A.B. *Animal Models for Therapeutic Strategies*. Insight Pharma Reports 2010. Cambridge Healthtech Institute.  
[Pubmed](#) | [Crossref](#) | [Others](#)
12. Meng, X.J. Discoveries of Animal Strains of Hepatitis E Virus: Implications for Animal Models and Zoonosis. *American College of Veterinary Pathologists and American Society for Veterinary Clinical Pathology*, Ithaca, New York, USA. (2004)  
[Pubmed](#) | [Crossref](#) | [Others](#)
13. St. George-Hyslop P.H., Westaway, D.A. Antibody clears senile plaque. (1999) *Nature* 400: 116–117.  
[Pubmed](#) | [Crossref](#) | [Others](#)
14. Connan, F., Campbell, I.C., Katzman, M., et al. A neurodevelopment model for anorexia nervosa. (2003) *Physiol and Behav* 79(1): 13–24.  
[Pubmed](#) | [Crossref](#) | [Others](#)
15. Iversen, S.D. Is it possible to model psychotic states in animals? (1987) *J Psychopharmacol* 1(3): 154-176.  
[Pubmed](#) | [Crossref](#) | [Others](#)
16. Castagne, V., Moser, P.C., Porsolt, R.D. Preclinical behavioural models for predicting antipsychotic activity. (2009) *Adv Pharmacol* 57: 381-418.  
[Pubmed](#) | [Crossref](#) | [Others](#)
17. Pippin, J. *Humane Seal Fact Sheet on Animal Experimentation*. PCRM (2009).  
[Pubmed](#) | [Crossref](#) | [Others](#)
18. Immunodeficient rodents opening new doors for investigators. *Animal Health Research reviews*. (1996) 1: 1–8.  
[Pubmed](#) | [Crossref](#) | [Others](#)
19. Pinkert, C.A. The history and theory of transgenic animals. *LAN*. (1997) 26: 29-34.  
[Pubmed](#) | [Crossref](#) | [Others](#)
20. Yan, Y., Panos, J.C., McCormick, D.J., et al. Characterization of a novel H2A (–) E+ transgenic model susceptible to heterologous but not self thyroglobulin in autoimmune thyroiditis: Thyroiditis transfer with Vbeta8+ T cells. (2001) *Cell Immunol* 212: 63-70.  
[Pubmed](#) | [Crossref](#) | [Others](#)
21. Grune, B., Fallon, M., Howard, C., et al. Report and recommendations of the international workshop “Retrieval approaches for information on alternative methods to animal experiments”. (2004) *ALTEX* 21(3): 115-127.  
[Pubmed](#) | [Crossref](#) | [Others](#)
22. Centre for Alternatives to Animal Testing. *Johns Hopkins University. AltWeb: Databases*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
23. Australian and New Zealand Council for the Care of Animals in Research and Teaching. (2007) *ANZCCART Fact Sheets*.  
[Pubmed](#) | [Crossref](#) | [Others](#)
24. Ensembl. *Ensembl Genome Browser*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
25. International Mouse Strain Resource. *IMSR: International Mouse Strain Resource*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
26. National Institutes of Health. *Mouse Models of Human Cancers Consortium*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
27. Festing MFW. *Isogenic info*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
28. UK Coordinating Committee on Cancer Research. *UKCCCR guidelines for the welfare of animals in experimental neoplasia (2nd edition)*, London. (1997)  
[Pubmed](#) | [Crossref](#) | [Others](#)
29. USDA. *National Agricultural Library. Animal Welfare Information Center: AWIC*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
30. ILAR. *ILAR: Institute for Laboratory Animal Research, Animal models and strains*. (2007)  
[Pubmed](#) | [Crossref](#) | [Others](#)
31. Kilkenny, C., Parsons, N., Kadoszewski, E., et al. Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. (2009) *PLoS One* 4(11): 7824.  
[Pubmed](#) | [Crossref](#) | [Others](#)
32. Festing, M.F., Altman, D.G. Guidelines for the design and statistical analysis of experiments using laboratory animals. (2002) *ILAR Journal* 43(4): 244-258.  
[Pubmed](#) | [Crossref](#) | [Others](#)
33. Kilkenny, C., Browne, W.J., Cuthill, I.C., et al. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. (2010) *PLoS Bio* 8(6): 1000412.  
[Pubmed](#) | [Crossref](#) | [Others](#)
34. Schulz, K.F., Altman, D.G., Moher, D. CONSORT Statement: updated guidelines for reporting parallel group randomised trials. (2010) *BMJ*. 2010 340: 332.  
[Pubmed](#) | [Crossref](#) | [Others](#)

35. Rollin, B.E. Toxicology and new social ethics for animals. (2003) *Toxicol Pathol* 31: 128-131.  
[Pubmed](#) | [Crossref](#) | [Others](#)
36. Committee for Medicinal Product for Human Use, 1997. Replacement of Animal Studies by in vitro Models (CPMP/SWP/728/95).  
[Pubmed](#) | [Crossref](#) | [Others](#)
37. Russell, W.M.S., Burch, R.L. *The Principles of Humane Experimental Technique*. Methuen: London; 1959.  
[Pubmed](#) | [Crossref](#) | [Others](#)
38. Committee for Medicinal Products for Human Use. Concept paper on the Need for Revision of the Position on the Replacement of Animal Studies by in vitro Models (CPMP/SWP/728/95). 2011; EMA/CHMP/SWP/169839/2011.  
[Pubmed](#) | [Crossref](#) | [Others](#)