International Journal of Science, Strategic Management and Technology
Volume **02** Issue **02** February-2026 | ISSN: **3108-1762 (Online)** | Impact Factor: 3.8
An International, Peer-Reviewed, Open Access Scholarly Journal Indexed in recognized academic databases

"A Review of Alternative Methodologies: Reducing Animal use in Scientific Research"

Corresponding Author : **Ms.Shraddha Pramod Ubale**

Affiliation : Postgraduate student

Institution : Konkan Gyanpeeth Rahul Dharkar College of Pharmacy, Karjat , Maharashtra 410201.

Author : **Dr. Vanita Kanase**

Affiliation : Principal

Institution : Aims college of pharmacy, Aims foundation, Vadvali, Dombivali (East) – 421 204

https://doi.org/10.55041/ijsmt.v2i2.026

Cite this Article: Kanase, S. P. U. ,. V. (2026). "A Review of Alternative Methodologies: Reducing Animal use in Scientific Research". International Journal of Science, Strategic Management and Technology, *Volume 10*(01). https://doi.org/10.55041/ijsmt.v2i2.026

ABSTRACT:

As medical technology has progressed in both research and development, more animals have been employed in studies.Every year, millions of animals are utilized in experiments all around the world. However, these models come at a high cost, require a lot of work, and sometimes produce results that cannot be applied to a human in vivo setting. They have also brought up important social and ethical problems recently. This has compelled the researchers to look for methods to shorten the duration of drug screening procedures, as well as to reduce the quantity of animals used and improve the treatment of animals humanely. Several alternatives to animal testing have been proposed in order to address the drawbacks of the practice and avoid the unethical aspects of it. Three R's have been used by Russell and Burch to define these alternatives: reduction, refinement, and replacement. Physico-chemical procedures, tissue culture, microbiological systems, stem cells, DNA chips, microfluidics, computer analysis models, epidemiological surveys, and materials derived from plant tissue are some of these alternative methods. In this review, a brief description of these alternatives and their benefits is discussed.

Key words: Alternatives to animal testing, Animal welfare, In silico models, Imaging techniques, Ethical considerations

INTRODUCTION:

Animal studies primarily aim to develop new treatments for infectious and non-infectious diseases through drug testing and toxicological screenings. Animals help researchers to understand the effects of medical procedures and surgeries. Additionally, they produce vaccines, antibiotics, and other products for diagnostic and treatment purposes 1,2.. In clinical testing laboratories, animals are separated from their groups and used as tools, regardless of their natural instincts. Whole animals or their organs and tissues are used in experiments. Animals are euthanized using established methods. Often, animals that survive clinical testing are euthanized at the end of the experiment to prevent pain and distress . Animals may die during experiments, such as LD 50 analysis.3.

For an extended period, environmentalists and animal lovers have expressed concern over the use of animals in scientific research and biological testing. Numerous legislative measures have been put forth in this regard to ensure that animals are treated humanely and to restrict animal research.4,5. At the EU level, there is a continuous effort to find alternatives to animal testing whenever possible. Creating techniques that employ fewer animals or injure them less is encouraged when replacement is not an option. The 3Rs Principle (Replacement, Reduction, and Refinement) was introduced in 1959 by Russel and Burch and is thought to be a useful guide for ethical animal testing practices. This principle not only promotes alternative approaches to animal testing, but also aims to improve animal welfare and minimize suffering when necessary. This is significant because the main argument against animal use among institutional figures, activists, and the general public is based on suffering rather than painless killing.6. The seventh amendment to the Cosmetics Directive (76/768/EEC) was approved by the European Union in 2003. The EU document aims to eliminate animal testing for cosmetic products by 2009, regardless of the availability of substitute models. Since then, scientists in Europe and around the world have worked together to develop and validate alternatives to using animals whenever possible. 7. To create safe products for humans, animals, and the environment, it's important to understand the effects of chemicals on various organisms and how they vary depending on developmental stage, health status, and genetic composition. A chemical's ability to cause an effect depends on its concentration-time profile (internal exposure) and inherent activity (toxicity). Data on certain chemicals may only be available for specific species and exposure scenarios.8. To reduce animal suffering and stop unethical animal testing, several laws have been passed. In 1824, the Royal Society for the Prevention of Cruelty to Animals established an organization to promote animal rights. In 1876, the UK passed the Animal Cruelty Prevention Act. 9. Balls (1994) reports that in 1876, the UK passed an act to prevent animal cruelty. It originated in India, France, and the United States in 1960, 1963, and 1966, respectively. Currently, international regulations protect animals from cruelty and misuse. Organizations such as ICH (International Conference on Harmonization of Technical Requirements for Pharmaceutical Registration for Human Use), CCSEA (Committee for Control and Supervision on Experimental Animals), NIH (National Institute of Health), and OECD (Organization for Economic Cooperation and Development) provide guidelines for animal care, breeding, feeding, transportation, and use in scientific research.10.

THE CONCEPT OF REDUCTION, REFINEMENT, REPLACEMENT(3R's):

In a public meeting, Charles Hume and William Russell first discussed the Three Rs (reduction, refinement, and replacement) as a method to eliminate inhumanity in animal experimentation. This Symposium and Russell and Burch's book, The Principles of Humane Experimental Technique were initiated by UFAW in 1954 with the advice of a distinguished committee chaired by Peter Medawar. 9. This approach encourages using fewer animals, resulting in a "reduction" in the total number of animals used in an experiment. Animal experiments require careful planning and refinement to minimize pain and distress. Higher animals should also be "replaced" by smaller organisms and different approaches.

Reduction:

Reduction refers to methods for using fewer animals to obtain comparable levels of information or for maximizing the information obtained from a given number of animals (while causing no additional pain or distress) in order to eventually use fewer animals to obtain the same scientific information. This method relies on controlling environmental variability in animal housing and study areas, incorporating newer technologies, analyzing experimental design, and employing appropriate statistical techniques.13

Refinement:

Refinement is the process of making adjustments to experimental or husbandry practices in order to improve the welfare of the animals and reduce or eliminate their suffering. IACUCs should be aware that certain study types may have unanticipated or planned experimental outcomes that cause pain, even though institutions and investigators should make every effort to eliminate pain and distress through refinement. The study's objectives may dictate the elimination or retention of these outcomes.1

Replacement:

Replacement methods do not involve the use of animals. The term refers to both absolute (replacing animals with inanimate systems like computer programs) and relative (replacing vertebrates with animals lower on the phylogenetic scale).13

ALTERNATIVE PROCEDURES:

Microorganism based:

Studies on toxicology and carcinogenesis—the process by which cancer is produced—often employ these. These are predicated on a chemical's ability to cause mutations in a cell's DNA, which serves as the genetic information hub of the cell. An example of this would be the Ames Test, which is capable of identifying 80–90% of all chemicals that have been linked to cancer. Its main function is screening, and it needs to be validated by animal experiments. Using fungi can reduce the need for laboratory animals.4,14.

Software Model:

Drugs' molecular structures can be designed by computers to target particular receptors. For instance, because an HIV treatment was desperately needed, human tissue cultures and computer models were used to test protease inhibitors for patients instead of animals. Since the data from animals was inconclusive, Roche Pharmaceuticals was able to get approval for a new heart medication in 1997 based on data from a virtual heart. In silico, or on a computer, scientists can complete experiments in a matter of minutes that would take months or years to complete in a lab or clinic. Animal studies must be used to validate this technique before it can be used in place of animals.15

Organ/Tissue culture technique:

These tests involve the use of human and normal animal tissues and bodily fluids in both in vitro and in vivo settings to measure the absorption, distribution, and biotransformation of various drugs. For example, human dopaminergic neurons can be used in place of animal models of Parkinson's disease and transgenic models with altered PARK gene expression. Percutaneous absorption and the everted sac technique are two examples of in vitro techniques used to test for absorption. Rat gut technique and solid dosage form disintegration are two examples of in vivo methods. Although autoradiography is an in vivo procedure, methods such as protein binding models and ultrafiltration can be used to do distribution testing in vitro. Subcellular fraction, isolated hepatocytes, and isolated liver perfusion are examples of in vitro biotransformation tests. In vivo approaches include radiolabelled studies and clinical drug metabolism investigations.16.

Alternate Species:

Many limitations on the experimental use of higher model vertebrates, such as guinea pigs, rats, dogs, monkeys, etc., have been brought about by ethical concerns. As a result, it has been proposed to employ alternative organisms. Animals used in experiments are substituted with other model organisms.25

Alternate species	**Their use**	**Reasons**
Zebra fish(*Danio rerio*)	Developmental biology, Genetics, Drug testing	Transparent embryo
Fruit fly (*Drosophila melanogaster*)	Genetics, Neurobiology, Developmental studies	Short life cycle, Simple genome
Nematodes (*Caenorhabditis elegans*)	Genetics, Neurobiology, and Developmental biology	Simple anatomy and well-mapped genome
Yeast (*Saccharomyces cerevisiae*)	Molecular biology and genetics	Serves as model
Tadpoles (*Xenopus laevis*)	Developmental biology and neurobiology studies	Large eggs and embryo

Bacterial Systems (e.g., *Escherichia coli*)	Molecular biology for cloning and protein expression studies	
Sea Urchins (e.g., *Strongylocentrotus purpuratus*)	Developmental biology to study fertilization, cell division, and early embryonic development	Transparent embryo and ease of manipulation
Frogs (e.g., *Xenopus*)	Developmental biology and toxicology	Large eggs and ease of manipulation
Invertebrates (e.g., *Aplysia californica*)	Neurobiology research to study learning and memory	Simple nervous system.

Fig 1. Alternate species with their use and reasons

Information exchange:

To minimize the need for extra animals in an experiment, the amount of data gathered per animal should be maximized. Examples include the use of some imaging modalities that allow longitudinal measurements in the same animal (rather than culling cohorts of animals at specific time points), as well as Blood microsampling, which enables repeated sampling in the same animal with tiny volumes. In these cases, it is critical to balance the reduction in the number of animals used against any additional suffering that may result from their continued use. Sharing data and resources (e.g., animals, tissues, and equipment) among research groups and organisations can also help with reduction.

Human tissue:

Human tissues from patients with Alzheimer's and Parkinson's diseases have been used in research. Treatment for HIV/AIDS has been developed through human studies and human tissue, especially blood. It is ethically possible to test new medications on human tissues after receiving full informed consent. Many researchers even employ human tissue since it is more suitable than animal tissue and because these conditions are unique to people.18

DNA Chips:

These make pharmacogenetics research possible, which aids in individualized medication therapy. Glass slides studded with a variety of genes or DNA fragments are known as DNA chips. After being exposed to a novel medication, a DNA sample labeled with fluorescent dyes is cleaned over the chip. The colors show which genes have been activated or suppressed by the experimental medication when the genes on the chip bind together with the DNA in the sample. This method aids in the creation of medications for specific individuals.19

Stem cells:

Stem cells have the ability to differentiate along diverse lineages and self-renew. Adult stem cells (ASCs) are derived from adult tissues, whereas embryonic stem cells (ESCs) are found in the embryo. induced pluripotent stem cells (iPSCs) are reprogrammed from adult somatic cells by overexpressing some important transcription factors, such as Oct4, Klf4, Sox2, c-myc, Nanog, and Lin28. ESCs are pluripotent cells that have the capacity to differentiate into three layers of embryonic germ cells in addition to their ability to self-renew.20.

Human Volunteers:

Participating humans can serve as a viable substitute for animal testing models in other research domains, including nutrition, drug abuse, and pain management. Human volunteers have a major benefit over animal participants because they may interact with researchers and supply more information as the study progresses. In contrast to animal testing, human volunteers who donate healthy and compromised tissues through surgery provide a more appropriate method of studying human biology and disease. To replace rabbit irritation tests, skin and eye models made of reconstituted human skin and other tissues have been developed. By donating tissue, both living and deceased donors increase the number of human

samples available for research while decreasing the number of animal subjects required. Previously, post-mortem brain tissue provided.21

Tissue Bioprinting:

Three-dimensional (3D) tissue bioprinting is a revolutionary scientific advancement in drug discovery and development that employs novel assay models to better predict drug effects on humans. These tissue models, which mimic the characteristics of live human tissues, are created on microplates to assess the toxicity and efficacy of small molecules or other therapeutics. By combining tissue engineering, stem cell research, disease biology, and in situ detection devices for tissue characterization and drug development, 3D tissue bioprinting creates disease-relevant tissue models that can close the predictability gap between the results of current 2D cell-based assays and human testing. 21

Emerging imaging technologies:

magnetic resonance spectroscopy (MRS), positron emission tomography (PET), magnetoencephalography (MEG), magnetic resonance imaging (MRI), functional MRI (fMRI), and
techniques that provide a view of the human body, in particular the brain, that cannot be obtained by studying animals include transcranial magnetic stimulation (TMS), event-related optical signals (EROS), PET, and single-photon emission computed tomography (SPECT). 4

CONCLUSION:

This review focuses on alternatives to animal testing, emphasizing the principles of reduction, refinement, and replacement as guiding frameworks for ethical research practices. Advances in technology, such as in silico models, tissue cultures, and novel imaging techniques, present promising avenues for obtaining reliable results without the ethical concerns associated with animal experimentation.
By supporting these novel methodologies, researchers can improve scientific rigor while reducing animal suffering. Furthermore, regulatory efforts and public awareness have accelerated the shift towards more humane research practices. The scientific community must continue to advocate for and invest in these alternatives to ensure that future research is ethical and responsible, ultimately benefiting both human health and animal welfare. Scientists, ethicists, and Lawmakers will continue to collaborate.

CONFLICT OF INTEREST :

None

REFERENCES:

1. Giacomotto, J., & Segalat, L. (2010). High-throughput screening and small animal models: Where are we? *British Journal of Pharmacology, 160*(2), 204–216.
2. Hendriksen, C. F. (2009). Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement. *Expert Review of Vaccines, 8*(3), 313–322.
3. Rusche, B. (2003). The 3 Rs and animal welfare—Conflict or the way forward. *ALTEX, 20*, 63–76.
4. Arora, T., Mehta, A. K., Joshi, V., Mehta, K. D., Rathor, N., Mediratta, P. K., & Sharma, K. K. (n.d.). Substitute of animals in drug research: An approach towards fulfillment of 4R's. *Indian Journal of Pharmaceutical Sciences*. Retrieved from http://www.ijpsonline.com
5. Richmond, J. (2002). Refinement, reduction, and replacement of animal use for regulatory testing: Future improvements and implementation within the regulatory framework. *ILAR Journal, 43*(Suppl), S63–S68.
6. Freires, I. A., Sardi, J. C. O., de Castro, R. D., & Rosalen, P. L. (2017). Alternative animal and non-animal models for drug discovery and development: Bonus or burden? *Pharmaceutical Research, 34*(4), 681–686. https://doi.org/10.1007/s11095-016-2069-z
7. Adler, S., Basketter, D., Creton, S., Pelkonen, O., van Benthem, J., Zuang, V., et al. (2011). Alternative (non-animal) methods for cosmetics testing: Current status and future prospects—2010. *Archives of Toxicology, 85*, 367–485.

8. Madden, J. C., Enoch, S. J., Paini, A., & Cronin, M. T. D. (2020). A review of in silico tools as alternatives to animal testing: Principles, resources and applications. *ATLA: Alternatives to Laboratory Animals, 48*(4), 146–172. https://doi.org/10.1177/0261192920965977
9. Balls, M. (1994). Replacement of animal procedures: Alternatives in research, education and testing. *Laboratory Animals, 28*, 193–211.
10. Doke, S. K., & Dhawale, S. C. (2015). Alternatives to animal testing: A review. *Saudi Pharmaceutical Journal, 23*(3), 223–229. https://doi.org/10.1016/j.jsps.2013.11.002
11. Rollin, B. E. (2003). Toxicology and new social ethics for animals. *Toxicologic Pathology, 31*, 128–131.
12. Ranganatha, N., & Kuppast, I. J. (2012). A review on alternatives to animal testing methods in drug development. *International Journal of Pharmacy and Pharmaceutical Sciences, 4*, 28–32.
13. National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press.
14. Zurlo, J., Rudacille, D., & Goldberg, A. M. (1983). *Animals and alternatives in toxicity testing*. London: Academic Press.
15. Roncaglioni, A., & Benfenati, E. (2008). In silico-aided prediction of biological properties of chemicals: Oestrogen receptor-mediated effects. *Chemical Society Reviews, 37*(3), 441–450.
16. Piersma, A. H. (2006). Alternative methods for developmental toxicity testing. *Basic & Clinical Pharmacology & Toxicology, 98*(5), 427–431.
17. National Centre for the Replacement, Refinement & Reduction of Animals in Research. (n.d.). *The 3Rs*. https://nc3rs.org.uk/who-we-are/3rs#:~:text=Sharing
18. Festing, M. F. (1994). Reduction of animal use: Experimental design and quality of experiments. *Laboratory Animals, 28*(3), 212–221.
19. Nuwaysir, E. F., Bittner, M., Trent, J., Barrett, J. C., & Afshari, C. A. (1999). Microarrays and toxicology: The advent of toxicogenomics. *Molecular Carcinogenesis, 24*(3), 153–159.
20. Kim, T.-W., Che, J.-H., & Yun, J.-W. (2019). Use of stem cells as alternative methods to animal experimentation in predictive toxicology. *Regulatory Toxicology and Pharmacology, 105*, 15–29. https://doi.org/10.1016/j.yrtph.2019.03.016
21. Kaylor, A. (2023). Alternatives to animal testing models in clinical and biomedical research. *Feature.*
22. Liebsch, M., Grune, B., Seiler, A., et al. (2011). Alternatives to animal testing: Current status and future perspectives. *Archives of Toxicology, 85*(8), 841–858. https://doi.org/10.1007/s00204-011-0718-x
23. Gruber, F. P., & Hartung, T. (n.d.). Alternatives to animal experimentation in basic research.
24. Madden, J. C., Enoch, S. J., Paini, A., & Cronin, M. T. D. (2020). A review of in silico tools as alternatives to animal testing: Principles, resources and applications. *ATLA: Alternatives to Laboratory Animals, 48*(4), 146–172. https://doi.org/10.1177/0261192920965977
25. Hill, A. J., Teraoka, H., Heideman, W., & Peterson, R. E. (2005). Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicological Sciences, 86*(1), 6–19