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Animal research: Ethics, regulations, and alternatives

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Abstract
Researching and testing using animals have been a practice of scientists for a long time now, and it has proved beneficial, given the medical advancements that were possible due to it. Currently, animals are used in the areas of biological studies of a fundamental nature; research and development; quality control of products and devices for human medicine, dentistry, and for veterinary medicine; preclinical toxicological evaluation; and other safety evaluations of vaccines and antibodies. They are part of development mandatory requirement for testing drugs and medicines in pharma industry throughout the world. The animal experimentation laws, policies, and guidelines followed in countries differ and cover different animals. This review discusses the need for animal research, what for animals are used in research, alternatives to animal testing, and ethics and regulations related to animal research.

Keywords: Animal research, body on a chip, CPCSEA, drug testing, ethics, toxicology

Introduction
The use of animals in research and education dates back to the period when humans started to look for ways to prevent and cure ailments. Research on living animals has been practiced since ancient times. They are used as part of development mandatory requirement for testing drugs and medicines in pharma industry throughout the world and also for disease diagnosis, teaching, and training. A number of computer simulation and other models have been recommended for use as alternatives to use of animals for pharmacology education and research, but animal experimentation continues to remain crucial to a high proportion as there are no other alternatives to substitute biological system so far. Therefore, even today animals are indispensable in research aiming to improve the health of humans and animals [1].

What is animal research?
Animal research is a phrase that most people have heard but are perhaps still unsure of exactly what is involved. Whether it is called animal research, animal testing, *in vivo* testing, vivisection, or animal experimentation, it refers to the experimentation carried out on animals. It is used to assess the safety and effectiveness of everything from medication to cosmetics, as well as understanding how the human body works. "Vivisection," a term preferred by those who oppose the use of animals in research, means cutting into or dissecting a living animal. Researchers prefer to use the term "animal experimentation" [2, 3].

Which animals are used in research?
Many different species of animals are used for animal testing around the world. The most common animals used in experiments are mice, rabbit, guinea pig, sheep, albino rats, primates, mini-pigs, farm animals, frogs etc., but mice is a common animal model. Less frequently, birds and fish are used [4].

Why animals in research?
All mammals and all vertebrates have strong similarities because of evolutionary ties and common ancestry. Chimpanzees, our closest living animal cousins, share 98% of our human genes. Even mammals that look quite different from us share a large percentage of our genes; small and furry mice share 92% of our genes. Fruit flies, for instance, have their own version of approximately 44% of our human genes [5]. This genetic similarity has allowed researchers to use them as human models since human testing is not ethical and legal. Some animals have biological similarities to humans that make them particularly good models for specific diseases, such as rabbits for atherosclerosis or monkeys for polio.

While humans cannot be used in research for ethical reasons, there are also variables which can be controlled in a mouse (like diet, housing, clean air, humidity, temperature, and genetic makeup) could not be controlled in human subjects [6].
There are no good alternatives to animal testing, because alternatives do not do a sufficient job of making sure products and medicines are safe. A living body is an extraordinarily complex system. It is not possible to reproduce a beating heart in a test tube or a stroke on a computer. While we know a lot about how a living body works, there is an enormous amount we simply do not know: the interaction between all the different parts of a living system, from molecules to cells to systems like respiration and circulation, is incredibly complex. Even if we knew how every element worked and interacted with every other element, which we are a long way from understanding, a computer has not been invented that has the power to reproduce all of those complex interactions – while clearly you cannot reproduce them all in a test tube.
Since all modern biotechnology derived pharmaceuticals exert highly human specific pharmacodynamics properties, there is a need to study the safety in a species closely related to humans for legislative marketing authorization. As per statutory requirements also, these products will have to be evaluated for safety, efficacy, and toxicity in rodents and non-rodents prior to phase I and phase II clinical trials.
Most people believe that in order to achieve medical progress that will save and improve lives, limited and very strictly regulated animal use is justified. It is worth noting that animal research benefits animals too: more than half the drugs used by vets were developed originally for human medicine. Some of the most important drugs and treatments that we use today are based on animal testing. Insulin, vaccines, antibiotics, and numerous cancer and HIV treatments were developed through the use of animals in research [7]. Because of our ability to manipulate the genome of the laboratory mouse, the mouse has become the surrogate for human disease [8].

What are animals used for in research?
There are four main reasons why animals are used in research:

To advance scientific understanding
Basic biological research helps us understand how living things work. Many basic cell processes are the same in all animals, and the bodies of animals are like humans in the way that they perform many vital functions such as breathing, digestion, movement, sight, hearing, and reproduction. To treat disease, doctors and scientists must understand how the healthy body works. This, in turn, leads to an understanding of what happens to the body when we fall ill and how this can be put right. A great deal of the knowledge of the body's anatomy and functions can be traced to scientific findings from animal research. Even simple animals can be used to study complex biological systems such as the nervous or immune systems, which follow the same basic organization and function in all animals. For example, much has been learnt about the function of neurons from studying the giant squid axon. Information from this sort of work can then be applied to higher animals and humans. Basic research is important because it involves the study of how organisms function and develop. The learning associated with basic research is intricately linked to applied or practical research although it is more academic in nature [9].

As models to study disease
Humans and animals share hundreds of illnesses, and consequently animals can act as models for the study of human illness. For example, rabbits suffer from atherosclerosis, as well as diseases such as emphysema, and birth defects such as spina bifida. Dogs suffer from cancer, diabetes, cataracts, ulcers, and bleeding disorders such as hemophilia, which make them natural candidates for research into these disorders. Cats suffer from some of the same visual impairments as humans. Studying disease mechanisms in animal models leads directly to the development of new technologies and medicines that benefit both humans and animals. An important area of biomedical research is the use of animal testing to study numerous diseases. By analyzing animal models, scientists can learn what causes disease as well as how it develops and what aspects of genetics, the environment, or diet contribute to the development of disease [4].
Animals which are altered to create models of disease are known as induced models. For example, surgery which damages a particular section of the spinal cord in rats gives rise to symptoms like those seen in human patients with similar spinal cord damage. These animal models help researchers understand what happens in the body following this type of damage and have been used in the development of new therapies. Recent advances in genetic technology have allowed the development of transgenic animals, which have new genes inserted into their DNA, allowing them to develop human diseases which do not naturally affect them. In particular this has allowed mice to model many human diseases which were previously difficult to study.

To develop and test potential forms of treatment
Once researchers learn more about a particular disease, animals are used to develop and test these potential therapies as part of the applied research process. For example, medicines for Parkinson's disease have been developed using animal models with induced Parkinson's-like symptoms. Models such as these are an essential part of applying biological research to real medical problems, allowing new targets for disease intervention to be identified. Data from animal studies is essential before new therapeutic techniques and surgical procedures can be tested on human patients.
Diagnostic tools such as scanners, and implants such as heart pacemakers or artificial hips, are safe and effective only because they were developed and tested in animals. Many surgical techniques, such as open heart surgery and heart transplants, rely on methods and equipment that were developed using animals.

To protect the safety of people, animals, and the environment
New medicines require testing because researchers must measure both the beneficial and the harmful effects of a compound on a whole organism. A medicine is initially tested *in vitro* using tissues and isolated organs, but legally and ethically it must also be tested in a suitable animal model before clinical trials in humans can take place. Drug testing is one area of biomedical research that utilizes animal testing. How drugs are metabolized is vital to ensuring the safety of medications [7]. The animal tests provide data on efficacy and safety. They not only identify potential safety concerns but also determine the doses which will be given to volunteers and patients during the first human trials.
Testing on animals also serves to protect consumers, workers, and the environment from the harmful effects of chemicals. All chemicals for commercial or personal use must be tested so that their effect on the people and animals exposed to them is understood.

Animals are used in the testing of drugs, vaccines and other biologics, and medical devices, mainly to determine the safety of the medical product. For drugs and biologics, the focus of animal testing is on the drug's nature, chemistry, and effects (pharmacology) and on its potential damage to the body (toxicology).

For medical devices, the focus of animal testing is on the device's ability to function with living tissue without harming the tissue (biocompatibility). Most devices use materials such as stainless steel or ceramic that we know are biocompatible with human tissues. In these cases, no animal testing is required. However, some devices with new materials require biocompatibility testing in animals.

Toxicology testing is another important aspect of animal testing because it is used to ensure the safety of various substances such as drugs. These tests are conducted by pharmaceutical corporations and other establishments such as animal testing facilities who are hired to perform animal testing for private companies. Toxicology testing is an area of animal testing with approximately a million animals used per year in Europe alone. This type of testing comprises an estimated ten percent of all animal testing procedures that occur each year. Roughly five thousand animals undergo testing for just a single chemical and the number is more than double that for pesticides [7].

Toxicology testing has many benefits, which include assessment of products such as: pesticides, food products, additives, chemicals, and pharmaceuticals. Animals may receive just one application of a substance or they may receive regular or repeated applications over the course of several months. For some animals, they receive the chemical for their entire lifespan [7]. The types of animal tests currently performed are: eye irritancy, acute toxicity, repeated dose toxicity, skin corrosivity/irritation, skin sensitization, pharmacokinetics/toxic kinetics and metabolism, dermal penetration, mutagenicity, carcinogenicity, reproductive and developmental toxicity, neurotoxicity, ecotoxicity, and pyrogenicity [4].

These tests are extremely expensive, costing in the millions and taking several years to finish. Some of the tests last a mere month while others continue for several months or more. These tests, due to the nature of the products, are the most rigid. They assess overall toxicity to the body of the animal as well as any damage to eyes or skin. Other aspects include the potential to cause malignant growth or affect reproduction[7].

Animal Research and Drug Safety

Animal research is a central pillar of drug development. Animal testing forms a big part of pharmaceutical research. Currently, before a new compound or drug can even enter clinical trials on humans, it has to be tested on non-human animals. Pharmaceutical companies need to prove that the drug is "safe" before human trials can be initiated, and this is done using non-human animals. Animals are necessary and used in pharmaceutical testing for numerous reasons. One such reason is determining whether the drug is toxic to the subject. This is done by investigating how the compound is broken down, or metabolized, by the liver into other byproducts known as metabolites. These metabolites can build up over time with repeated drug use and often cause serious and unpredictable problems. It would be exceedingly unwise and very dangerous to give a human a drug that has not undergone rigorous animal testing. It is important to realize, however, that what happens in an animal model may not mirror what will happen in a human. Animal testing is still limited in what it can tell us. But as it stands there is currently nothing available that can give us as much information on how a particular drug will interact with our bodies as an animal model can [10]. At the same time, the Declaration of Helsinki says that it is unethical to give experimental treatments to humans that have not been tested first in laboratory animals.

Animal experiments are not used to show that drugs are safe and effective in human beings – they cannot do that. Instead, they are used to help decide whether a particular drug should be tested on people. Animal experiments eliminate some potential drugs as either ineffective or too dangerous to use on human beings. If a drug passes the animal test it is then tested on a small human group before large scale clinical trials [11].

Statistics of Animals Used In Research

While animal research is conducted in most countries in the world, it is hard to come up with any accurate worldwide figures. The USA counts only warm-blooded animals in research, teaching, and testing except for rats, mice, and birds that were bred for research. The EU counts all vertebrates as well as cephalopods (octopuses, squid, etc.). Currently, no country counts all invertebrates (e.g., fruit flies and nematode worms). Only a small proportion of countries collect and publish data regarding their use of animals for testing and research, but it is estimated that more than 115 million animals – including mice, rats, birds, fish, rabbits, guinea pigs, farm animals, dogs, cats, and non-human primates – are used in laboratory experiments each year around the world. Not only is there considerable variation in how animals are used, but there is variation in how many and what types of animals are used in experiments. While experiments of vertebrates are regulated in most countries, those on invertebrates are not, and hence their accurate usage statistics are lacking [12].

The top 10 animal testing countries in the world are the USA, Japan, China, Australia, France, Canada, the UK, Germany, Taiwan, and Brazil [13]. In 2016, America is the leading country for the number of animals used in research (20 million), followed by China (16 million) and European Union (11.5 million) [14]. It is important to note that this statistics does not include rats, mice (which make up about 90% of research animals), birds and fish, as these animals are not covered by the Animal Welfare Act in the USA. Most procedures have been carried out on mice and rats (96%). Other animals used commonly include guinea pigs (22.3%), rabbits (17%), and hamsters (12.5%). In the UK, over the last ten years for which data are publicly available (2007–2016), mice were the most commonly used animal, appearing in 72.8% of experimental procedures. After mice, the most prevalent animals used were fish (13.6%), rats (6.3%), and birds (3.9%). These four constitute 96.6% of research procedures. Experimental procedures involving specially protected species (i.e., horses, dogs, cats, and non-human primates) accounted for 3.4%.

The approximate total number of animals used in research in Australia in 2016 was over 9 million [15]. The total number of animals used for scientific research in EU member countries in 2014 was 12.8 million with France, Germany, and the United Kingdom being the top three animal using countries in EU [16].

While most of the above-mentioned animals are used in India too, their accurate usage statistics is lacking. In India, the

National Centre for Laboratory Animal Sciences (NCLAS), Hyderabad, supplies approximately 50,000 animals to laboratories every year and to 175 institutions in India, including pharmaceutical companies and educational institutions [17]. The use of frogs in India needs special permission from the respective State Chief Wildlife warden, since the frogs are labeled as endangered species. Frogs are included under Schedule IV of the Wildlife Protection Act 1972 of India as well as under the red list of International Union for the Conservation of Nature and Natural Resources [18, 19]. As per CPCSEA guidelines amendment in 2006, a separate permission from CPCSEA is required to carry out any experiments on large animals. However, the Institutional Animal Ethics Committee (IAEC) of the respective establishments is empowered to permit experiments on small animals [18, 20]. The number of animal used in teaching varies from 1% to 10% of total animals used [21] Most of the animals used are small rodents. These are mostly used for fundamental biological research and breeding purposes [22].

Animal Research and Ethics

"The question is not, can they reason? Nor, can they talk, but can they suffer."

This profound thought provoking statement was made by Jeremy Bentham to oppose the ideology of many scientists in the nineteenth century that animals are incapable of suffering from pain [23]. Animals have the right to the respect of their dignity and, hence, the respect of their species-specific characteristics, needs, and behaviors. If human beings fail to respect the acknowledged dignity of animals, they abuse their freedom and fail to respect their own dignity. Animal ethics are not stringent rules mandating researchers to conduct animal research in certain ways, but an arena for promoting the expression of human moral obligations toward animals used in research. Despite Greek and Roman references to animal experimentation by Aristotle (4th century BC) [24], Erasistratus (3rd century BC) [24], and Galen (2nd century AD) [25], the earliest reference to animal welfare and ethics occurs only in the 19th century [26]. For example, what we know as Society for Prevention of Cruelty to Animals (SPCA) today was originally organized in England in 1822 [26]. In 1831, the first seeds were sown for today's animal ethics guidelines by Marshall Hall, a British physiologist [26].

In 1876, the English House of Commons passed the first bill relating to animal experimentation (the Cruelty to Animals Act 1876 = An Act to Amend the Law Relating to Cruelty to Animals 1876) following which, a number of countries including USA followed suit [26]. In 1959, William Russell, an intelligent young zoologist; and Rex Burch, a microbiologist, published "The Principles of Humane Experimental Technique." Therein, they categorized humane animal experimentation techniques under replacement, reduction, and refinement, now referred to as the 3Rs – Replacement, Reduction, and Refinement [27]. To this, the present day animal activists have added another "R"– Rehabilitation. Animal experimentation ethics did not emerge de novo. It evolved over centuries of philosophical traditions [28]. And the regulatory bodies came into existence only after the pressure from the animal protection groups and public. These regulatory groups enacted laws to regulate the care and usage of laboratory animal models. At present, animal welfare became an important concern while doing experimental studies on animals. Many of the regulatory bodies are showing concern about the animal welfare which in turn provides guidelines for proper animal care and usage during research [29].

Scientists should ensure that all individuals who use animals under their supervision receive explicit instruction in experimental methods and in the care, maintenance, and handling of the species being studied. Refining the experimental procedures themselves and refining the management of pain are the most important issues that researcher should be aware of. They should carefully assess the method of administration, the effects of the substance on the animal, and the amount of handling and restraint required. Therefore, adequate training is an important aspect of the refinement of animal research, and should continually be reviewed and improved [23]. Humane consideration for the well-being of the animal should be incorporated into the design and conduct of all procedures involving animals [30].

Animal Research Laws and Regulations

There was little public objection to animal experimentation until the 19th century, when the increased adoption of domestic pets fueled interest in an anti-vivisection movement, primarily in England. This trend culminated in the founding of the Society for the Protection of Animals Liable to Vivisection in 1875, followed by the formation of similar groups [31, 32]. Queen Victoria was an early opponent of animal testing in England, according to a letter written by her private secretary in 1875: The Queen has been dreadfully shocked at the details of some of these [animal research]practices, and is most anxious to put a stop to them" [33]. Soon the anti-vivisection campaign became strong enough to pressure lawmakers into establishing the first laws controlling the use of animals for research: Great Britain's Cruelty to Animals Act of 1876 [34]. In 1959, The Principles of Humane Experimental Technique by zoologist William Russell and microbiologist Rex Burch was published in England where the "Three Rs" were explained which formed the basis for many international animal welfare laws [35].

A public outcry over animal testing and the treatment of animals in general broke out in the United States in the mid-1960s, leading to the passage of the AWA. An article in the November 29, 1965, issue of *Sports Illustrated* about Pepper, a farmer's pet Dalmation that was kidnapped and sold into experimentation, is believed to have been the initial catalyst for the rise in anti-testing sentiment [36]. Pepper died after researchers attempted to implant an experimental cardiac pacemaker in her body [37].

Animal testing in the United States is regulated by the federal Animal Welfare Act (AWA) and the PHS Policy on Humane Care and Use of Laboratory Animals. AWA was passed in 1966 and amended in 1970, 1976, and 1985 [38]. The AWA defines "animal" as "any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm blooded animal." The AWA excludes birds, rats, and mice bred for research, cold-blooded animals, and farm animals used for food and other purposes [39].

The AWA requires that each research facility develop an internal Institutional Animal Committee (more commonly known as an Institutional Animal Care and Use Committee, or IACUC) to "represent society's concerns regarding the welfare of animal subjects." Along with the AWA, facilities using live vertebrate animals in research funded by the US Public Health Service (PHS) must also adhere to the Public Health Service Policy on Humane Care and Use of Laboratory Animals (the PHS Policy) and follow the Guide

for the Care and Use of Laboratory Animals produced by the Institute for Laboratory Animal Research (ILAR).

Across the EU, the care and use of laboratory animals in research is regulated under a common law frame (Directive 2010/63/EU). The EU directive puts more emphasis on species-specific education and training in laboratory animal science and the implementation of the 3R's in every aspect of care and use of laboratory animals.

Currently in India, experimentation on animals is covered under the provisions of Prevention of Cruelty to Animals Act, (PCA Act) 1960 and the Rules under the amended Act of 1998 and 2001. This is implemented through a committee called "Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA)." It is a statutory body which was established in 1964 under Section 15(1) of Chapter 4, of the PCA Act under the Ministry of Environment Forest & Climate Change. The CPCSEA provides guidelines for performing experiments on animals and maintenance of animal house [40]. The registration of animal house is mandatory with CPCSEA and is to be renewed every 3 years. At present, in India, there are 1723 facilities registered with CPCSEA for conducting experiments using animals. Besides the rules and procedures laid down by the CPCSEA, the Indian National Science Academy (INSA) and Indian Council of Medical Research (ICMR) have also formulated certain guidelines for care and use of animals in scientific research as well as in medical colleges [41, 42].

In 2003, the Pharmacy Council of India (PCI) issued a directive to all pharmacy schools in India to use CAL software in place of classroom animal experiments [43]. Also in 2003, MCI's Executive Committee concluded: "As an alternative to these tests involving animals, JIPMER, Pondicherry, has developed EX-PHARM Blank CD. This CD has been specially prepared as a 100% replacement to animals used in undergraduate courses in Medicine, Pharmacology, and Veterinary Science" [44]. More recently in 2011, the University Grants Commission issued guidelines to phase out dissection of and experimentation on live animals in zoology and life science courses [45].

Since 2013, India has banned the testing of cosmetic products sold in the country, making it the first country in South Asia to do so. Not only this, India has also banned the import of cosmetics that have been tested on animals abroad! India has also banned animal-testing of soaps and detergents manufactured in India. In April 2016, Union Minister Maneka Gandhi along with the Indian Ministry of Health and Family Welfare established the prohibition of animal testing for household products manufactured in India. Similarly, European Union, Norway, and Israel have also banned cosmetic testing in animals [46]. However, the Chinese government requires animal testing on certain cosmetics that are produced domestically (such as hair coloring and sunscreen) and requires animal tests for all imported cosmetics.

While animals are used extensively in drug research, mere use of animals to study or practice surgical techniques has been restricted. Nearly 95 per cent of medical schools in North America – including Yale, Harvard and Stanford – do not use any animals to train medical students, and experience with animal dissection or experimentation on live animals is not required or expected of those applying to medical school. In the United Kingdom, it is against the law for medical (and veterinary) students to practice surgery on animals [48].

Regulation of animal testing will not only reduce the usage of animals but also help in assessing the data of already done research or helping in harmonizing the test methods. Animal testing is a necessary evil; it is indispensable and inevitable. It should be viewed in a broader perspective in the larger interest of a nation's progress. Laws and regulatory practices of the ethics committees should be strictly enforced [49].

Alternatives to Animal Research

Cell lines that have been harvested from animal or human tissue are often used and then bred further in laboratory culture. These experimental methods outside the organism – "*in vitro* methods"– are of major importance and are widely used to elucidate cellular processes or the effect of medications on cell metabolism. Significant methodological progress has been made in recent decades in working with *in vitro* systems. Especially in drug testing and the development of pharmacological substances, they have helped to reduce the use of laboratory animals. However, they cannot completely replace animal experiments as the complexity of the entire organism – i.e., the interaction between organs and tissues – cannot be fully reproduced in these isolated, artificial systems. Furthermore, producing and growing organ and cell cultures requires the killing of animals.

Another method for avoiding experiments on live animals comes from regenerative medicine and is known as "body on a chip." This method was developed from tissue engineering or bioprinting, whereby replacement organs for humans are grown from human tissue and created using a 3D printer. These mini-organs are placed on a microchip and supported by an artificial maintenance system. Sensors on the microchip measure certain parameters, such as organ temperature and oxygen content, and record changes in the system. The "body on a chip" method is used for testing the toxicity or pharmacological properties of biological and chemical substances.

"In silico methods" (in silico = on a computer) are also becoming increasingly important as alternatives to animal experimentation. These computer-controlled analysis and simulation techniques are used for assessing risk when researching tolerance to substances or theoretically modeling life processes, among other purposes. As a result, experimental data in neurobiology is increasingly being entered in computer models to portray and predict the functions of the central nervous system. Computer simulation is also used in higher education to visualize complex biological relationships.

Instructional videos on animal experimentation serve as teaching aids to prepare learners for the work with living animals and promote a responsible approach. In spite of favorable aspects, these alternative methods have a severe disadvantage. The human or animal body possesses more than 200 different cell types whose interactions are coordinated in organs and tissue structures. To study this complexity is an important part of biological research, and this can only be done on an intact organism. Even if a drug appears to be useful during its development in cell culture, it may turn out to be inactive or even toxic in other cell types, or lead to the formation of breakdown products in the body, causing damage in other organs. Conversely, a substance may be inactive in cell culture but effective in an intact organism. For example, without using animals, the synthetic antibiotic Prontosil® would never have been discovered in the 1930s. While Prontosil® showed no effect in the cell culture, it was proven that it has very strong antibacterial properties in a

living organism, and Gerhard Domagk was awarded the Nobel Prize for this discovery in 1939 Rimentation [50].

In recent past, several non-animal test methods have been formally validated and accepted by some countries as replacements for an existing animal test. Examples include [17]

1. IC50 test – which can be used to determine the cytotoxicity of a chemical in terms of the chemical's ability to inhibit the growth of half of a population of cells.
2. EpiSkinÂ® test – alternative to Draize test that uses test-tube sized models of human skin.
3. The 3T3 Neutral Red Uptake Phototoxicity Test – uses cells grown in culture to assess the potential for sunlight induced ("photo") irritation to the skin.
4. An embryonic stem cell test – using mouse-derived cells to assess potential toxicity to developing embryos has been validated as a partial replacement for birth-defect testing in rats and rabbits.
5. EpiDerm™ test – replacement for skin corrosion studies in rabbits.
6. Human Microdosing – can provide information on the safety of an experimental drug and how it is metabolized in the body by administering an extremely small one-time dose.
7. Compu Series – developed and sold by the Chennai-based Blue Cross. It allow students to digitally dissect everything from "Compufrogs" and "Compurats" to "Compuroaches."
8. TOPKAT (TOxicity Prediction by Computer Assisted Technology) – software package available in India, allows researchers to predict chemicals' oral toxicity as well as their degree of skin and eye irritation.
9. CD developed by JIPMER – to replace all animals used in undergraduate courses in pharmacology, medicine and veterinary science.
10. QSARs (Quantitative Structure/Activity Relationship programs) – These are computer programs which can predict the toxicity of new chemicals or drugs based on their similarity to more established compounds.
11. Silicon chip technology – This technology allows rapid identification of genes whose activity changes in response to certain diseases and drugs.

Both in basic research and in application-oriented research, alternative methods and animal experiments are used in a complimentary manner. While individual molecular and cellular aspects of life processes are researched *in vitro* or in silico as much as possible, scientific work on animals is irreplaceable in order to further our understanding of complex relationships within the entire organism. By carefully considering and selecting the most suitable methods, we have the chance to significantly reduce the number of test animals and the stress caused to them.

Conclusion

Testing plays a crucial part in medical research; it is necessary for understanding and sorting out problems that we human beings are vulnerable to. Worldwide, it is reckoned that every second approximately three animals are used for the experimentation process. Progressive changes have been made in recent years in the principles and practice of animal testing. Opponents to any kind of animal research believe that animal experimentation is cruel and unnecessary, regardless of its purpose or benefit. There is no middle ground for these groups; they want the immediate and total abolition of all animal research. If they succeed, it would have enormous and severe consequences for scientific research. In fact, animal research has contributed to 70% of Nobel prizes for physiology/medicine. While a combination of newer *in vivo* and *in vitro* techniques do provide viable and cost‑effective alternatives to certain pharmacodynamic and pharmacokinetic evaluations of drugs, animal testing is yet required for repeat dose toxicity, carcinogenicity of drugs, and certain behavioral studies. Hence, it appears that animals are not entirely dispensable, especially in research, which reiterates the need to practice the principles of 4Rs in animal experimentation and intensify our efforts in developing and validating suitable alternatives to their use.

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