Original Article

Use of Animals in Research: Do we have any Alternatives?

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ABSTRACT

There has been a rampant increase in the use of animals for biomedical and other research during the recent past. The animals (both rodents and non-rodents) invariably form a part of the experiments related to research in science in general and biomedicine in particular. The purists believe that it is essential to perform experiments in animals to ensure the safety and assess the feasibility of experimenting in human beings. The ethicists insist that the use of animals in research should be minimized and every effort should be made to ensure that ethical treatment is meted out to these humble creatures. On the other hand, the revolutionists are of the opinion that animals hardly serve any good purpose in research and they can be conveniently replaced by other technological advancements.

Keywords: Research, Animal Experiments, Toxicity, In-vitro studies, Clinical research.

INTRODUCTION

To prevent affliction of unnecessary pain and suffering to animals, the Central Government of India constituted a Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which was duty bound to take all such measures as may be necessary to ensure that animals are not subjected to unnecessary pain or suffering before, during or after the performance of experiments on them. For this purpose, the Government made "Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998" as amended during 2001 and 2006, to regulate the experimentation on animals. "As a follow up of the recommendations of the CPCSEA, which was constituted under the provisions of Section 15 of the Prevention of Cruelty to Animals Act (1960), the Union Ministry of Environment and Forests (MoEF) banned the use of live animals in dissection and other experiments in educational and research institutions. But scientists conducting new molecular research were kept exempted from the ban" (The Times of India: April 17, 2012). However, there was clash of ideas at the World Laboratory Animal Day (April 24)

celebrations during national symposium on 'Laboratory animal science in the new millennium - challenges and solutions', organized by the National Centre for Laboratory Animal Sciences, NIN, ICMR, and Laboratory Animal Science Association of India at the National Institute of Nutrition (NIN) wherein DG-ICMR and Secretary, Department of Health Research, Government of India, Dr. V M Katoch, during his speech, criticised the opposition to use of animals in medical research, whereas, the animal rights activist Amala Akkineni of Blue Cross of Hyderabad and Federation of Indian Animal Protection Organizations was equally vehement in her opinion against the practice & said, in course of time, the use of laboratory animals should be completely stopped and pharmaceutical companies and other research organizations should make use of technology to carry out drug trials and other such researches. Dr. B Sesikeran, the Director NIN said that over a period of time, the use of laboratory animals for research should be minimized. Anjani Kumar, Director, Animal Welfare Board of India (AWBI) and member-secretary, CPCSEA said a circular had been issued to avoid use of live animals for experiments up to under-graduate level by the government (The Times of India-Hyderabad-April 25, 2012).

Use of Animals in Research – Global Scenario

Every year around the world, nearly 100 million animals are bred, injected, infected, cut open, genetically altered, force-fed drugs / chemicals and ultimately killed for scientific research, testing and education. The British Union for the Abolition of Vivisection (BUAV) and Dr. Hadwen Trust suggests that nearly 115 million vertebrate animals may be used worldwide each year. The top 10 countries are United States, Japan, China, Australia, France, Canada, United Kingdom, Germany, Taiwan and Brazil[1]. As per a report of the European Commission, in 2008, over 12 million animals were used-France; UK and Germany were the highest users of animals. Also, the 27 countries in the EU reported that they used 21,315 dogs, over 330,000 rabbits, over 9,500,000 rodents, over 750,000 birds and over 9,000 primates[2]. As per reports from the Great Britain, more than 3.6 million animals were used in over 3.7 million experiments in the UK in 2010- an overall increase of 37% from the year 2000. The use of animals in experiments during the year 2011included 2,679,763 mice, 271,535 rats, 11,537 guinea pigs,15,461 rabbits, 4,552 dogs,235 cats, 8,380 horse & other equids, 4,340 pigs, 37,714 sheep, 1,62,618 birds, 383 reptiles, 15,915 amphibians, 5,63,903 fish and 2,720 primates[3].

Use of Animals in Research – Indian Scenario

In India, one of the largest animal suppliers, 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. Every year many vivisectors come to India because, in their own countries, they cannot get away with doing the type of animal testing they can here. The poor animals are even poorer in India as in recent past only, the UK-based National Anti-Vivisection Society (NAVS) issued a report on Indian animal testing based on a review of Indian research papers in the international scientific literature and CPCSEA's inspections of 467 laboratories wherein, NAVS found key faults in the animal testing industry in India and concluded that years of scientific research in India have been invalidated by poor scientific procedure, poor laboratory practices and a lack of appropriate animal care. A few years ago, People for Ethical Treatment to Animals

(PETA) and the CPCSEA rescued a monkey named 'Paro' and 36 others from Pune's National Institute of Virology (NIV) after uncovering horrid conditions. Unable to provide even one record for any of the animals it used, NIV had confined most of its monkeys to tiny cages for more than a decade, and some had been disfigured or paralyzed from confinement and abuse. Some monkeys were missing fingers and teeth, while others – who had gone insane from years of intensive confinement – spun in circles around their cages. In June 2002, members of the CPCSEA inspected the dog-housing facilities of Delhi's Ranbaxy Laboratories and found that most of the animals were suffering from dermatitis, infectious diseases and defects that resulted from inbreeding. These instances of pathetic state of poor martyrs (animals) raised concerns regarding the quality of research being done in the country.

Are Animals a Replica of Humans?

The basic question that whether the experiments conducted in animals can be replicated and the results reproduced in human beings-has started intriguing a cult of researchers. Especially in the arena of new drug development, it has been proposed at several forums that animal experiments constitute a very inefficient means of developing new human clinical interventions, and were insufficiently reliable when predicting human toxicity. Their sensitivity to a wide range of toxins was generally accompanied by poor human specificity, severely limiting the predictive value of positive test results (Knight 2008b). Also, the animal model of biomedical research is radically misguided, because animals are significantly different from humans in ways that affect the metabolism and elimination of tested drugs and thereby their effectiveness and the side effects they may or may not produce. The result is that every year millions of people across the globe become sick, and hundreds of thousands die, because of unforeseen reactions to prescribed medications that were approved as safe on the basis of animal testing[4,5]. The New York Times dated February 11, 2013 reported "For decades, mice have been the species of choice in the study of human diseases. But now, researchers report evidence that the mouse model has been totally misleading for at least three major killers- sepsis, burns and trauma. As a result, years and billions of dollars have been wasted following false leads". The most significant trend in modern research in recent years has been the recognition that animals are rarely good models for the human body. Studies have shown time and again that researchers often waste lives – both animal and human – and precious resources by trying to infect animals with diseases which they would not normally contract. Dr. Richard Klausner of the US' National Cancer Institute admitted, "The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades, and it simply didn't work in humans". There are many examples of drugs, such as monoclonal antibodies and neurotherapeutics that show dramatically different effects in humans and animals. In many cases, not only does animal testing hurt animals and waste money, it also harms and kills humans. For example, Thalidomide, Zomepirac (Zomax) , Rofecoxib (Vioxx), Rimonabant and Diethylstilboestrol (DES) were all tested on animals and judged safe, but they had devastating consequences for the people who used them.

Human Being- The Most Appropriate Model!

Almost all important developments in health are attributable to human studies, including anaesthesia; bacteriology; germ theory; the stethoscope; morphine; radium; penicillin; artificial respiration; antiseptics;

the CAT, MRI and PET scans; the discoveries of the relationships between cholesterol and heart disease, between smoking and cancer and between diet and other illnesses; the development of X-rays and the isolation of the virus which causes AIDS. Animal testing played no role in these and many other similar developments. Clinical trials, the use of human volunteers, case studies, autopsy reports and statistical analyses permit far more accurate observation – as well as the use of actual environmental factors related to human disease. It is widely agreed that comparative studies of human populations allow doctors and scientists to discover the root causes of human diseases and disorders so that preventive action can be taken. By the early 1940s, human clinical investigation strongly indicated that asbestos causes cancer. However, animal studies repeatedly failed to demonstrate this, and proper workplace precautions were not instituted in the U.S. until decades later. Similarly, human population studies have shown a clear risk from exposure to low-level ionizing radiation from diagnostic X-rays and nuclear wastes, but contradictory animal studies have stalled proper warnings and regulations. Likewise, while the connection between alcohol consumption and cirrhosis is indisputable in humans, repeated efforts to produce cirrhosis by excessive alcohol ingestion have failed in all nonhuman animals except baboons, and even the baboon data is inconsistent. Many other important medical advances have been delayed because of misleading information derived from animal models. The animal model of polio, for example, resulted in a misunderstanding of the mechanism of infection. Studies on monkeys falsely indicated that the polio virus was transmitted via a respiratory, rather than a digestive route. This erroneous assumption resulted in misdirected preventive measures and delayed the development of tissue culture methodologies critical to the discovery of a vaccine. While monkey cell cultures were later used for vaccine production, it was research with human cell cultures which first showed that the polio virus could be cultivated on non-neural tissue. Similarly, development of surgery to replace clogged arteries with the patient's own veins was impeded by dog experiments which falsely indicated that veins could not be used. Also, the Epidemiological studies led to the discoveries of the relationship between smoking and cancer and to the identification of heart disease risk factors'[6] whereas, conversely, tobacco company executives relied on misleading animal-based studies to deny the link between smoking and cancer as recently as 1994[7]. Population studies demonstrated the mechanism of the transmission of AIDS and other infectious diseases and also showed how these diseases can be prevented, whereas animal studies have produced no real results in terms of preventing or treating AIDS[8]. The National Institutes of Health have reported that more than 80 HIV/AIDS vaccines that have passed animal testing have failed in human clinical trials[9]. Probably, that is why it is being said "When it comes to testing HIV vaccines, only humans will do"[10].

A New Outlook

In 1959, British zoologist William Russell and British microbiologist Rex Burch published '*The Principles of Humane Experimental Technique'*. This work introduced the goals of Replacement, Reduction, and Refinement (3Rs): Replacement of animal testing with other techniques, Reduction of the number of animals tested, and Refinement of animal tests to reduce suffering. To this, the present day Animal activists have added another 'R'- Rehabilitation. This pioneer work set the stage for consideration for instituting ethical treatment to animals used in research and the need to explore suitable alternatives. The

basic principles postulated more than five decades ago are of immense relevance even today. Replacement means replacing 'higher' animals with 'lower' animals. Microorganisms, plants, eggs, reptiles, amphibians, and invertebrates may be used in some studies to replace warm-blooded animals[11]**.** Alternately, live animals may be replaced with non-animal models, such as dummies for an introduction to dissection for teaching the structure of the animal or the human body, mechanical or computer models, audiovisual aids, or *in vitro* modeling[12]. Reduction means minimizing the number of animals needed to perform an experiment or teach a concept. Methods to achieve this include: performing pilot studies to determine some of the potential problems in an experiment before numerous animals are used, designing a study to utilize animals as their own controls' and gathering a maximum amount of information from each animal, and perhaps gathering data for more than one experiment concurrently. Refinement means refining experimental protocols to minimize pain and / or distress whenever possible. Rehabilitation as per the Prevention of Cruelty to Animals (PCA) Act 1960 means- 'take care after experimentation'.

Alternatives to Animal Experimentation

The alternatives to animal testing are primarily based on biochemical assays, experiments in cells that are carried out in-vitro, and computational models and algorithms. These techniques are typically far more sophisticated and specific than traditional approaches to testing in whole animals, and many in vitro tests are capable of producing information about the biological effects of a test compound that are equally accurate and in some cases more accurate than the information collected from studies in whole animals. Basic research these days is focusing increasingly on developing models based on organisms that are less expensive and more experimentally efficient than mammals. Such organisms include fruit flies (*Drosophila melanogaster*), nematodes (*Caenorhabditis elegans*), and zebra fish (*Brachydanio rerio*). 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 the following:

1. Traditional toxicity tests performed on animals are becoming outmoded. These tests result in the deaths of many animals and often produce data that are irrelevant to humans. An example of a toxicity test in animals that is being replaced by in vitro techniques is the LD50 test, in which the concentration of a chemical is increased in a population of test animals until 50 percent of the animals die. A similar in vitro test is the 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. Another example of a toxicity test performed on animals that often produces inaccurate results is the Draize test, in which a chemical, such as a cosmetic or pharmaceutical agent, is applied to the skin or eye of a rabbit to study the toxicity of a chemical to human skin. The European Union recently approved a replacement for the Draize test called the EpiSkinÂ® test developed by L'Oreal and IMEDEX, which is an in vitro method that uses test-tube sized models of human skin.
3. Pharmagene Laboratories, based in Royston, England, is the first company to use only human tissues and sophisticated computer technology in the process of drug development and testing. With tools from molecular biology, biochemistry, and analytical pharmacology, Pharmagene conducts extensive studies of

human genes and how drugs affect those genes or the proteins they make.

4. The U.S. National Disease Research Interchange provides human tissue to scientists investigating diabetes, cancer, cystic fibrosis, muscular dystrophy, glaucoma, and other human diseases. *In vitro* genetic research has isolated specific markers, genes, and proteins associated with Alzheimer's disease, muscular dystrophy, schizophrenia, and other inherited diseases.
5. A 3-dimensional model of breast cancer has recently been developed that will allow investigators to study the earliest stages of breast cancer and test potential treatments. Rather than studying cancer in rodents, this model, which uses both healthy and cancerous human tissue, effectively allows the study of cancer as it develops in humans[13].
6. The 3T3 Neutral Red Uptake Phototoxicity Test uses cells grown in culture to assess the potential for sunlight-induced ("photo") irritation to the skin. Also, 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[14].
7. Human skin model tests are now in use, including the validated EpiDerm™ test, which has been accepted almost universally as a total replacement for skin corrosion studies in rabbits[15].
8. The use of human skin leftover from surgical procedures or donated cadavers can be used to measure the rate at which a chemical is able to penetrate the skin.
9. 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 that is well below the threshold necessary for any potential pharmacologic effect to take place[16].
10. The majority of medical schools in the U.S., including Harvard, Stanford, and Yale, have replaced their use of live animals in physiology, pharmacology, and/or surgical-training exercises with humane and effective non-animal teaching methods, including observation of actual human cardiac bypass surgery, patient simulators, cadavers, sophisticated computer programs, and more.
11. In addition to being more humane, non-animal teaching tools such as computer simulations, multimedia CD-ROMs, and models are also more economical than traditional animal-based teaching exercises. One popular alternative, the Compu Series, developed and sold by the Chennai-based Blue Cross, allow students to digitally dissect everything from "Compufrogs" and "Compurats" to "Compuroaches".
12. The National Cancer Institute (NCI) now uses human cancer cells – taken by biopsy during surgeries – to perform first-stage testing for its new anti-cancer drugs. This practice spares the lives of the millions of mice whom the institute previously used every year and gives the institute a much better shot at combating against cancer.
13. US-based Physiome Sciences has developed software programmes which simulate the human body's organs and processes. These software programmes are so advanced that they can be used to predict the effects of drug therapies for a variety of diseases.
14. TOPKAT (TOxicity Prediction by Computer Assisted Technology) a software package available in India, allows researchers to predict chemicals' oral toxicity as well as their degree of skin and eye irritation. Faster, cheaper and more accurate than animal tests, TOPKAT is now used by the Food and

Drug Administration (FDA) and the Environmental Protection Agency (EPA) in the US as well as by the US Army. Also available in India is a CD developed by JIPMER which has been specially designed and prepared to replace all animals used in undergraduate courses in pharmacology, medicine and veterinary science.

15. Many companies around the world choose to subject animals to painful tests like eye irritancy and lethal dose tests in which cosmetics / personal care products are dripped into their eyes, smeared on their skin, sprayed in their faces or forced down their throats. Instead of measuring how long it takes a chemical to burn away the cornea of a rabbit's eye, manufacturers can now drop that chemical onto donated human corneas. The Irritation Assay System, a simple test-tube procedure, spares millions of animals from horrific eye- and skin-irritation tests. Human skin cultures can also be grown and ordered for irritancy testing.
16. **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.
17. **Silicon chip technology-**This technology allows rapid identification of genes whose activity changes in response to certain diseases and drugs. It can help identify both whether a drug or chemical is going to be therapeutic or harmful.
18. **Cell cultures-**Almost every type of human cell can be grown in culture, although the cells behave more simplistically than in the living body. Cellular systems have been central to key research into cancers, sepsis, kidney disease and AIDS, and are routinely used in chemical safety testing, vaccine production, drug development and to diagnose disease.
19. **Human tissues-** Both healthy and diseased tissues can be donated from human volunteers after biopsies, surgery or death. Blood or urine samples can also be easily taken. Post-mortem brain tissue has provided important leads to understanding brain regeneration. Also, Reconstituted Human Epidermis (RHE) skin model (Trade names-Episkin, Epiderm and SkinEthic) from human skin derived from donated, unwanted skin from cosmetic surgery is being used to test the likely irritancy of chemicals and cosmetics to the skin.
20. **Volunteer studies-** These include Magnetic Resonance Imaging (MRI) which generates detailed pictures of the brain and, when used in conjunction with other techniques, can identify the location of specific brain activities, and Human Microdosing which involves giving very tiny doses of a chemical compound to human volunteers in order to monitor where it goes in the body.
21. **Population research-** Studying illnesses in human populations to understand the roles of genes, lifestyle, diet and occupation, has had a tremendous impact on saving lives, especially from cancer and heart disease.
22. **Epidemiological studies-** Results of epidemiological data collected over years (longitudinal studies) have provided researchers and health practitioners with the understanding of causes, treatments, and preventions of a range of human illnesses. Epidemiology is an extremely important method to identify risk factors for disease and to determine optimal treatment approaches to clinical practice, which typically will include lifestyle changes, and understanding of the role of genetics and potential environmental contributors to illness. Such studies only

revealed that smoking is associated with lung cancer, and it was the first area of research to identify AIDS when rare infections and malignancies surfaced in patients in the late 1970s.

Further, the famous Framingham Heart Study, ongoing for the last 60+ years, has given us more information about the causes, preventions, symptoms, and evidences of heart disease than any other single area of heart research. Some important conclusions drawn from the Framingham Heart Study- without doing any Animal experiments include:

- Learning that sleep apnea is tied to increased risk of stroke
- Pinpointing additional genes that may play a role in Alzheimer's
- Finding that fat around the abdomen associates with smaller, older brains in middle-aged adults
- Detecting that genes link puberty timing and body fat in women
- Determining that having a first-degree relative with atrial fibrillation is associated with increased risk for this disorder
- Discovering hundreds of new genes underlying the major heart disease risk factors—body mass index, blood cholesterol, cigarette smoking, blood pressure, and glucose/diabetes
- Identifying first definitive evidence that occurrence of stroke by age 65 years in a parent increases risk of stroke in offspring by 3-fold

Similar holds true for other such landmark studies like United Kingdom Prospective Diabetes Study (UKPDS), The Diabetes Control and Complications Trial (DCCT), etc.

23. ***In vitro* studies-** *In vitro* research and human cell cultures have proven superior to animal tests for a multitude of purposes. Some significant findings from *in vitro* testing include cancer-screening treatments, testing drugs with biochips, and replicating human skin for research. *In vitro* models of the brain and the blood-brain barrier are being used for studies of neurotransmitter pathways, electrophysiological characteristics, morphological associations of human diseases (i.e., Alzheimer's, Parkinson's, Huntington's, and epilepsy), new drug designs, receptor targets, and modes of action of new pharmaceuticals. Though surrounded by controversy because of potentially unethical procurement and uncompensated commercial use of the human cells, there are infinite possibilities of using human cell lines. For example, in 1951 Henrietta Lacks died of an aggressive form of cervical cancer. Researchers harvested her cells, called HeLa cells after (He)nrietta (La)cks, without family approval or knowledge.
24. **Clinical Studies-** The value of human volunteers in carefully designed and managed clinical studies can yield significant results without the use of animals, or harm to humans. Many individuals with both ordinary and terminal illnesses are willing to volunteer for new drug or treatment trials, or be part of a study collecting data on their illness. The numbers of ongoing human clinical studies testify to the fact that there is no shortage of volunteers. Studies with humans—both clinical non-invasive research performed with the highest ethical standards, and longitudinal epidemiological research—may in fact be two of the best alternatives to animals.
25. **Autopsies and Post-mortem studies-** Autopsy research has been responsible for the discovery and description of a number of diseases, including Legionnaire's disease, viral hepatitis, aplastic anemia,

and fetal alcohol syndrome. As a result of people donating their bodies to research, organ banks now exist, thereby giving researchers' an access to the supply along with detailed information about the person's medical history. McLean Hospital in MA, for example, houses the Harvard Brain Tissue Resource Center. First funded by the National Institutes of Health (NIH) in 1978, their "Brain Bank" is now the largest brain tissue research center in the world. It currently has over 6,000 donated human brain specimens, most from donors who had neurological disorders. The center serves as an important resource for studying neurological diseases like Alzheimer's, Parkinson's, etc.

26. **Computerized patient-drug databases and post-marketing surveillance-** Computer technology can collect detailed comprehensive records and maintain cross references on the side effects of drugs, treatments, etc. Once stored in a central database, researchers can rapidly identify dangerous drugs or interactions. Post-marketing surveillance of patients can also identify unexpected beneficial side effects. In fact, clinical observation of patient side effects led to the discovery of the anti-cancer properties of Nitrogen mustard and Actinomycin D, and the mood-elevating effects of Tricyclic antidepressants.
27. **Chromatography and spectroscopy-** These are physical and chemical techniques that identify, isolate, and measure compounds in drugs, toxins, and body fluids, such as blood, urine, or saliva. Effective use of these techniques may help in reducing the requirement of animals.
28. **Mathematical models and computer simulations-** Computer-based alternative methods produce computational disease and treatment models, collect and manage millions of human research data points, and carry out human clinical trials virtually. Computer model programs are able to simulate sophisticated anatomical functions such as heart rate and, along with other data, can be used to determine disease or predisposition to certain illnesses. For example, computer simulations of cancer cells are now used to test drug targets within them, and "mathematical models have helped to further our understanding of HCV [hepatitis C] dynamics and clinical trial results in humans."
29. **Non-invasive imaging techniques-** Imaging technology such as the CT scan (computed tomography), MRI (magnetic resonance imaging), AMS (accelerator mass spectroscopy), MEG (Magneto-encephalography), DTI (diffusion tensor imaging), ultrasound, and nuclear imaging are all alternatives to utilizing unreliable animal models to produce results specific to humans. These non-invasive techniques allow very sophisticated, real-time measurements of associations between structure and function in humans and are accurate with resolutions possible down to single cells. These imaging options have had their most extensive applications in the neurosciences, allowing direct, noninvasive studies of human neurophysiology.

CONCLUSION

There exist two schools of thought on this issue-the 'Contemporary Scientists' who criticize the opposition to use of animals in medical research, and the 'Revolutionary Scientists' who are quite vehement in their opinion against the practice & say that in course of time, the use of laboratory animals should be completely stopped and pharmaceutical companies should make use of technology to carry out drug trials. The

former believe that it is the need of the hour to generate scientific evidence regarding the safety as well as efficacy profile of the trial drug by conducting the animal studies as per the existing Regulatory guidelines, whereas the latter are of the view that Animal experiments constitute a very inefficient means of developing new human clinical interventions, and are insufficiently reliable when predicting human toxicity as their sensitivity to a wide range of toxins is generally accompanied by poor human specificity. They propose that the animal model of biomedical research is radically misguided, because animals and humans are significantly different in the pathways that affect the metabolism and elimination of therapeutic agents. In the opinion of the author, it is high time that we have been sacrificing animals in the name of education and research-that too when most of the experiments give misleading inference.

The need of the hour is to explore further, to endeavor to innovate and utilize the already known alternatives available to minimize the animal experiments involving these innocent creatures and try to be HUMANE!

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