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Alternatives to animal testing: A review

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Abstract:

The figure of animals worn in research has augmented with the expansion of research and development in medical technology. The tenderness, agony experienced by the animals during scientific experiments has been an assessment issue for a long time. Various alternatives to animal testing were proposed to overcome the drawbacks allied with animal experiments. A approach of reduction, refinement and replacement is being applied for laboratory use of animals .To fulfil this goal a number of new techniques have been devised which are called 'Alternatives' for use of animals in research involving drugs. Different alternative methods validated by international regulatory authorities such as using blood from human volunteers to test for the presence of fever-causing contaminants in intravenous medicines can save hundreds of thousands of rabbits each year from traditional "Pyrogen" test. Each composed of artificial human skin can save thousands of rabbits each year from painful skin corrosion and annoyance tests. The Bovine Corneal Opacity and Permeability Test and Isolated Chicken Eye Test use eyes from animals slaughtered for the meat industry instead of live rabbits to detect chemicals and products that are severely irritating to the eyes. The Reduced Local Lymph Node Assay for skin allergy testing makes it possible to reduce animal use by up to 75 percent compared with traditional guinea pig and mouse tests. When testing to determine chemical concentrations that are deadly to fish and other aquatic life, use of the fish threshold method can reduce the numbers of fish used by at least 70 percent compared with standard test methods.

Introduction

Exploit of animals for an assortment of purposes like food, hauling, pets, sports, amusement and camaraderie is as old as the human beings itself. Utilization of animals for the function of investigation is one of the extended uses. Asorted animals like mice, rats, hamsters, rabbits, fishes (examples – zebra fish, trout), birds (mainly chicken), guinea pigs, amphibians (frogs), primates, dogs, cats and all that are being worn in delve into for a long time . Drug testing and toxicological screenings which are valuable in the development of new treatments for infectious and non-infectious diseases is the main purpose of such studies. Animals also serve as a tool to under-stand effects of medical procedures and surgical experiments. Moreover, they are used to obtain products like vaccines, antibiotics and so on which are used in diagnostics as well as treatments.[1]

The figure of animals used in research has gone up with the advancement in medical technology. Every year, millions of experimental animals are used all over the world. For example, in UK, 3.71 million animals were used for research in the year 2011 [2].

The total number of animals used in the USA in the year 2009 was estimated to be 1,131,076, while that in Germany reached up to 2.13 million in 2001 [3]. This huge inhabitants of experimental animals usually comes from the breeding centers positioned in an assortment of universities and national breeding centers. At few instances use of the wild animals such as monkeys and birds is also followed.

In clinical testing laboratories, animals are isolated from their groups and used as a tool irrespective of their natural instincts. Intended for the experimental events, either a whole animal or its organs and tissues are used. For this purpose animals are euthanized (killed) by established methods. Numerous times, the animals existing the clinical testing are euthanized at the end of an experiment to avoid the later pain and distress. In some cases (for example in LD 50 analysis) animals die as a result of the experiment.

The ache, agony and death experienced by the animals throughout scientific experiments have been a debating concern for a long time. Argument is that being alive, animals have the rights against pain and distress and hence, their use for experimentation is unethical and must be bunged . Assorted acts and laws have been passed to bring the control over unethical use of animals and diminish the pain to animals during experimentation. For example, in 1824, the association for animal rights was formed by the Royal Society for the preclusion of cruelty to animals. In

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1876, an act for preclusion of cruelty to animal was fashioned in the UK[4]. It came into subsistence in India, France and USA in the year 1960, 1963 and 1966, respectively. At present, many convention and acts are followed at the international level, to protect the animals against the cruelty and misuse. The organizations like 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) provide the guidelines for animal house keeping, breeding, feeding, transportation, and mainly for their use in scientific experiments [4]. Besides the major concern of ethics, few more disadvantages of animal experimentation are requirement of skilled manpower and time uncontrollable protocols. Furthermore, very high cost concerned in breeding, housing and lengthy protocols of animal experiments is another short. [5]

Three Rs: reduction, refinement and replacement
The word "alternative" is used to describe any change in an animal test that achieves one or more of the "three R's":
1. Replaces a procedure that uses animals with a procedure that doesn't use animals
2. Reduces the number of animals used in a procedure
3. Refines a procedure to alleviate or minimize potential animal pain
Alternatives to animal testing were anticipated to overcome some of the drawbacks allied with animal experiments and let alone the unethical procedures. A strategy of 3 Rs is being functional which stands for reduction, refinement and replacement of laboratory use of animals [6]. Dissimilar methods and alternative organisms are applied to implement this strategy.

The impression of replacement of animals was first discussed in 1957 by Charles Hume and William Russell at the Universities Federation for animal wel-fares (UFAW)[5].

Reduction
With the help of statistical prop up and vigilant selection of study design one can construct consequential scientific results of an experiment. For example, in vitro cell culture is a good way to screen the compounds at early stages. Use of the human hepatocyte culture gives the information about how a drug would be metabolized and eliminated from the body. Inclusion of such method in study design helps to eliminate unsuitable compounds in preliminary stages only and minimizes the use of animals in further tastings [7].

Refinement
Stirring the enclose environment by taking care of animals reduces the stress on animals. Scientists should refine the animal facility so that tenderness, embarrassment and agony during animal life and scientific procedures are reduced.

Replacement
Assorted alternatives to the make use of animals have been suggested, such as in vitro models, cell cultures, computer models, and new imaging/analyzing techniques [5]. The in vitro models provide the prospect to study the cellular response in a closed system, where the tentative conditions are maintained. Such models provide prelude information for outcome of an experiment in vivo. For example, computer models were worn to study the working of the heart and to select the potential drug candidates [8]. In many countries, in vitro cell cultures have replaced the skin irritancy test and Draize eye irritancy test and use of animals in those.

Emergent unconventional methods
An assortment of methods has been recommended to pass up the animal use in trialing. These methods endow with an substitute means for the drug and chemical testing, up to some levels. Compensation allied with these methods are, time competence, requires less man power, and cost value. These methods are described in detail as follows-

Computer models
Computers can help to understand the assorted basic ideology of biology. Specialized computer models and software programs help to design new medicines. Computer generated simulations are used to foretell the assorted doable biological and toxic effects of a chemical or potential drug candidate without animal dissection. Only the most promising molecules obtained from primary screening are used for in vivo experimentation. For example, to know the receptor binding site of a drug, in vivo experimentation is necessary. Software known as Computer Aided Drug Design (CADD) is used to foretell the receptor binding site for a potential drug molecule. CADD works to identify probable binding site and hence avoids testing of unwanted chemicals having no biological activity. Also, with the help of such software programs we can tailor make a new drug for the specific binding site and then in final stage animal testing is done to obtain confirmatory results [9]. Hence, the total number of experimental animals is lowered and the objectives of Russel and Burche's 3 Rs are achieved.

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Another admired tool is the Structure Activity Relationship (SARs) computer programs. It predicts biological activity of a drug candidate based on the presence of chemical moieties fond of to the parent compound. Quantitative Structure Activity Relationship (QSAR) is the mathematical description of the relationship between physicochemical properties of a drug molecule and its biological activity [10]. The activities like carcinogenicity and mutagenicity of a potential drug aspirant are well predicted by the computer database. The recent QSAR software shows more appropriate results while predicting the carcinogenicity of any molecule. The advantages of computer models over conventional animal models are the speed and relatively inexpensive procedures [11]. A very good example is a study [12] which assessed the effectiveness of computer models versus the traditional laboratory practices. Computer assisted learning (CAL) respectively. CAL is an interactive computer assisted learning (CAL) program without involvement of real experimental tools.

Cells and tissue cultures

Use of in vitro cell and tissue cultures which involves growth of cells outside the body in laboratory environment can be an essential unconventional for animal experiments. The cells and tissues from the liver, kidney, brain, skin etc. are removed from an animal and can be kept outside the body, in suitable growth medium, for few days to several months or even for few years.

Alternative organisms

The principled issues have posed many precincts over the experimental use of higher model vertebrates like guinea pig, rats, dogs, monkeys etc. Therefore, use of unconventional organisms has been proposed. Different model organisms are used to re-place experimental animals.

Lower vertebrates

Lower vertebrates are an attractive option because of the genetic relatedness to the higher vertebrates including mammals. Besides, there are less ethical problems involved in the experimental use of lower vertebrates.

Paradigm – Danio rerio commonly called as zebra fish, is a small freshwater fish with an approximate length of 2–4 cm. It has a nearly transparent body during early development, which helps easy visual access to the internal anatomy. The optical clarity allows direct observation of developmental stages, identification of phenotypic traits during mutagenesis, easy screening, assessment of endpoint of toxicity testing and direct observation of gene expression through light microscopy. Small size, short life cycle and high fecundity favor its laboratory use. The working space, cost of laboratory solutions, test chemicals and the manpower involved are reduced by opting D. rerio as an unconventional to animals [13]. Its embryos and larvae can be developed and used for testing in cell culture plates and Petri dishes. Whole genome sequence availability makes Zebra fish an attractive option for molecular and genetic research.

Invertebrates

Invertebrate organisms are widely used as an alternative for laboratory use of animals. They have been used to study of assorted diseases like Parkinson's disease, endocrine and memory dysfunction, muscle dystrophy, wound healing, cell aging, programmed cell death, retrovirus biology, diabetes and toxi-cological testing [14]. Invertebrates have an undeveloped organ system and do not have the adaptive immune system, which poses some limitations for their use in human diseases. However, they hold numerous benefits, such as a brief life cycle, small size and simple anatomy, so that a large number of invertebrates can be studied in a single experiment within a short period with less ethical problems. Their cost of housing is less compared to the animals. For example, thousands of flies could be accommodated in a sanctuary where only few mice can be kept[15].

Microorganisms

Paradigm – **Saccharomyces cerevisiae**

Brewing yeast, Saccharomyces cerevisiae is the most admired and vital model organism due to its rapid growth, ease of replicating and mutant isolation, dispersed cells, well defined genetic system and highly handy DNA transformation system. Yeasts can be grown in solid or liquid culture and inaccessible as colonies derived from a single cell on solid media. Once an alternative test has been developed by a scientist, it must be scientifically "validated," or evaluated in multiple laboratories to see if its results reliably predict outcomes in people. Validation is sometimes a frustratingly slow process, and the United States has unfortunately proved to be far slower at validating alternatives than the European Union. After an alternative has been scientifically validated, it is then up to government authorities to decide whether—and to what extent—they will accept the use of the alternative to replace, reduce or refine animal use. The opinions of government regulators strongly influence the extent to which private companies use available alternatives instead of traditional animal tests[16].

Effective Examples of Alternatives

Nearly 50 different alternative methods and testing strategies have been developed, validated and/or accepted by international regulatory authorities. These are a few examples:

•Using blood from human volunteers to test for the presence of fever-causing contaminants in intravenous

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medicines can save hundreds of thousands of rabbits each year from traditional "pyrogen" tests.
•The Bovine Corneal Opacity and Permeability Test and Isolated Chicken Eye Test use eyes from animals slaughtered for the meat industry instead of live rabbits to detect chemicals and products that are severely irritating to the eyes.

•The 3T3 Neutral Red Uptake Phototoxicity Test can replace the use of mice and other animals in the testing of medicines and other products for their potential to cause sunlight induced "photo-toxicity."[17].Reduced Local Lymph Node Assay for skin allergy testing makes it possible to reduce animal use by up to 75 percent compared with traditional guinea pig and mouse tests[18].

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