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Review Article

A REVIEW ON ALTERNATIVES TO ANIMAL TESTING METHODS IN DRUG DEVELOPMENT

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ABSTRACT

The origins of the concept of *alternatives to animal testing* in the 1950s, and the range of replacement alternative methods and progress toward their incorporation into fundamental and applied research, education are discussed. The three R's that is Replacement, Reduction, Refinement are defined. Importance and advantages of alternatives to animal testing methods are mentioned. Information is given about the institutions researching alternatives to animal testing and resources available to assist in searching for alternatives are listed. Ethical considerations on the alternative methods are also discussed. It is concluded that much greater effort should be put into overcoming the barriers to the acceptance of replacement alternatives which currently limit the contributions they have to make toward greater humanity and better biomedical science.

Keywords: Alternatives, Refinement, Inhumanity, In-vitro, In-silico.

INTRODUCTION

Discovery of new lead compounds for novel therapeutic targets is a multi-step process involving drug design, synthesis and its pharmacological screening[1]. Selection of an animal model is one of the most important steps in any of the experimental pharmacological study. Drug development mainly deals with 3 stages Stage I: Hit and lead compound development phase(Identification of lead compound amongst the million compounds). Stage II: Preclinical studies (invitro and invivo experiments). Stage III: Clinical studies (experiments in humans)[2].

Welfare implies both fitness and a sense of wellbeing. An animal's welfare should be considered in terms of five freedoms: Freedom from Hunger and Thirst, Freedom from Discomfort, Freedom from Pain, Injury or Disease, Freedom to Express Normal Behaviour, Freedom from Fear and Distress[3].

Replacement of animals is what most people think of when you say alternatives to animal testing. A more sophisticated concept of alternatives has been put forth by Russell and Burch in their book, *The Principles of Humane Animal Experimental Techniques*. They promote a definition of alternatives as "the three Rs-replacement, reduction, and refinement" which has become a pervasive theme in biomedical research today[4].

Origin of the concept of Alternatives

In 1954, Charles Hume, founder of the Universities Federation for Animal Welfare (UFAW) made an original proposal for the Three Rs to the UFAW to take in consideration alternatives for animal testing and change scientific study in laboratory animal experiments. It was at the Universities Federation for Animal Welfare's 1957 *Symposium on Humane Technique in the Laboratory* (UFAW1957) that the concept of alternative to animal testing as a means of removing inhumanity from animal experimentation was first discussed in depth at a public meeting, notably by Charles Hume and William Russell. Committee under the chairmanship of Sir Peter Medawar, the Nobel prize-winning immunologist, along with Christine Stevens, founder of the Animal Welfare Institute (AWI) in the U.S, and William Lane-Petter, the Secretary of the Research Defence Society of Great Britain provided financial support and managed the project to publish the concept of animal testing alternatives. The microbiologist R.L. Burch and the zoologist W.M.S. Russell were chosen to publish the work. "The Principles of Humane Experimental Technique" was published in London in 1959, and the book defined animal testing alternatives as "The Three R's: Refinement, Reduction, and Replacement[5, 6].

Laboratory animals most commonly are used in three main areas: biomedical research, product safety testing, and education. Biomedical researchers use animals in their efforts to understand the workings of the body and the processes of disease and health, and to develop new vaccines and treatments for various diseases. Industry uses animals to test the safety and effectiveness of a wide range of consumer products, including drugs, cosmetics, household cleaning products, pesticides, industrial chemicals, and more. Educational uses include dissecting earthworms or frogs in biology class, as well as advanced training in surgical techniques for veterinary and medical students. Scientists also study animals to learn more about a given species, its biology and behaviour. They may study animals as models of psychological or social behaviours. They may learn from the special skills or abilities of an animal as well. For example, Navy researchers have studied dolphin echolocation--their built-in biological sonar system--to improve the human-made sonar systems used on board ships[7].

The animals involved are kept in captivity, or they are subjected to pain or distress that is not a natural part of their environment. They will either die as a result of the experiment or be deliberately killed afterwards, often for post mortem examination. In the laboratory an animal may be poisoned; deprived of food, water or sleep; applied with skin and eye irritants; subjected to psychological stress; deliberately infected with disease; brain damaged; paralysed; surgically mutilated; irradiated; burned; gassed; force fed and electrocuted. The list reads like a catalogue of torture methods[7, 8].

Fund for the Replacement of Animals in Medical Experiments (FRAME) considers that the current scale of animal experimentation is unacceptable. However, it also recognises that immediate abolition of all animal experiments is not possible. Vital medical research must continue to find treatments for diseases which lessen the quality of human and animal life. New consumer products, medicines, and industrial and agricultural chemicals must be adequately tested in order to identify potential hazards to human and animal health, and to the environment[7].

Alternative methods fall into three broad categories. These are called the 3 Rs: Replacement, Reduction, and Refinement. Replacement is what most people think of when you say "alternatives to animal testing": the animals are replaced, either by methods that don't involve animals at all (absolute replacement) or by those that use only the cells or tissues of animals (relative replacement). Many replacement alternatives involve these *in vitro* ("in glass") techniques, where the studies are done with cells or tissues in culture. If the cells come from human beings, it's absolute replacement. If they come from animals, it's relative replacement[7].

Replacement also 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. 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[9].

Advantages to replacement include utilizing pre-existing knowledge for teaching, applying known principles to new systems to look for similarities, and using less expensive animals or models to screen large numbers of agents for toxicity or mutagenicity. *Disadvantages* to replacement chiefly stem from the fact that any models are dependent on pre-existing information. In a system as complex as a live organism, all of the variables in physiology and pathology are not known. Thus, any research on new biological processes must utilize a living organism at some point. Unfortunately, replacement isn't always an option[9, 7].

One example of a replacement alternative is no longer considered an alternative it has become the norm. Not too many years ago, if a woman wanted to find out if she was pregnant, she'd have to get a laboratory test that involved killing a rabbit. Now, she can buy a small kit over-the-counter that tests her urine for certain chemicals--the rabbits have been replaced[7].

Reduction means minimizing the number of animals needed to perform an experiment or teach a concept. Some important kinds of testing just can't be done without animals, at least at this time. In these cases, researchers still can work to reduce the number of animals used in a given study. With careful experimental design and sophisticated statistical techniques, it is often possible to use far fewer animals and still get valid results. 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. Gathering a maximum amount of information from each animal, perhaps gathering data for more than one experiment concurrently. Consulting with a statistician to use only the numbers of animals required to achieve significance. Minimizing variables such as disease, stress, diet, genetics, etc., that may affect experimental results Performing appropriate literature searches and consulting with colleagues to ensure that experiments are not duplicated. Using the appropriate species of animal so that useful data is collected, Replacement whenever possible[7, 4, 5].

Refinement means refining experimental protocols to minimize pain or distress whenever possible. For those animals that do undergo testing, scientists may refine their methods to lessen or eliminate pain, distress, or suffering and to make the animals more comfortable[7]. Examples of refinement include Identifying pain and distress and making plans for preventing or relieving it. Setting the earliest possible endpoint for the experiment. That is, if the necessary information can be gathered before the animal experiences any ill effects from the experiment, this should be defined as the endpoint and the animal subsequently euthanized. For example, if measuring toxicity of a compound or survival following implantation of a neoplasm, a pilot study may determine that once certain clinical signs are seen, or a tumor achieves a certain size, the time course until debilitation or death are predictable. Subsequent experiments may then utilize the earlier endpoint of tumor size or clinical signs of toxicity, rather than death as the endpoint. Receiving adequate training prior to performing a procedure. Using proper handling techniques for animals. Ensuring that drug doses are correct and that the drugs used are not expired. Ensuring that procedures to be performed on the animal are reasonable for that species. Using appropriate analgesics and anaesthetics for potentially painful procedures. Performing surgeries and procedures aseptically to prevent infection. Performing only a single major survival surgery on any one animal, whenever possible. Performing appropriate post-surgical care, including thermoregulation and fluid balance. There are several specific research techniques in common use that are often criticized for their potential for causing pain or distress to animals[7, 4].

Scientists at private companies, universities, and government agencies are developing new cell and tissue tests, computer models and other sophisticated methods to replace existing animal tests. When an alternative method is developed, it must undergo an internationally recognized validation process before being officially approved. This procedure is very complicated and usually takes more than ten years. The alternative methods developed must first be subjected to comparative tests in a number of laboratories (round-robin studies) to demonstrate that the results obtained carry as much weight as those of in-vivo studies, so that the alternative method provides an equivalent level of safety. The results of these studies are submitted to the responsible scientific committee for evaluation. Once the validity of the method has been recognized by the respected scientific committee, the Organisation for Economic Co-operation and Development (OECD) can then officially approve the alternative method and incorporate it into an OECD guideline [10].

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[11].

In-vitro methods

Cell culture can be an alternative to animal. Instead of using animals, Cell and tissue culture studies are used to screen for anti-cancer, anti-AIDS, and other types of drugs, and they are also a means of producing and testing a number of other pharmaceutical products, including vaccines, antibiotics, and therapeutic proteins[4]. For example, cultured cells have been developed to create monoclonal antibodies, prior to this production required animals to undergo a procedure likely to cause pain and distress. However, even though cell or tissue culture methods may reduce the number of experiments performed on intact animals, the maintenance of cells in culture normally requires the use of animal-derived serum. Although exact figures are difficult to obtain, some have estimated that one million fetal cows are sacrificed each year to obtain the world's supply of fetal bovine serum, used to grow cultured cells. cell and tissue cultures can be used to test product ingredients. Cell culture experiments can show the lowest concentration at which an ingredient causes damage to cells. The results enable conclusions to be drawn about the ingredient's compatibility with tissue. Cell cultures are now also used routinely to test substances for mutagenic properties. 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[4, 10].

Human skin equivalent tests can be used to replace animal-based corrosive and irritative studies. EpiDerm from Mattek and EpiSkin and SkinEthic RHE model two subsidiaries of L'Oréal, are derived from human skin cells which have been cultured to produce a model of human skin[10].

Corrositex is an invitro test that determines chemical corrosivity. This test replaces the rabbit test of dermal corrosivity by providing a reliable means of mimicking this test. The core technology of the Corrositex test is based upon a proprietary bio-membrane and chemical detection system which becomes colored when exposed to potentially corrosive substances. Rabbit testing takes several weeks to get results. Additionally the test is expensive and cruel. Simply put, the Corrositex test saves time and money over traditional rabbit testing[12].

A skinpatch test has been designed and is used in Canada to measure development of rashes, inflammation, swelling or abnormal tissue growth on human volunteers. Unlike corrosives, substances defined as irritants cause only reversible skin damage[13].

Another approach has been the development of test methods that use cultured human cells. Human epidermal keratinocytes have been cultured to mimic the human epidermis, and are used to measure skin irritation and dermal corrosion. This method has been accepted by the European Union, and is intended to replace the Draize rabbit skin irritation test[10].

In August 2010, OECD has published the Test Guideline 439 which describes the new procedure for in vitro hazard identification of irritant chemicals[14].

In the drug development process it is very important to screen the drug for gastrointestinal absorption. Conventionally, it is a very lengthy and time-consuming process. Moreover this process also requires a large number of animals. Colon cancer cell lines (CaCo) grow confluently and form a monolayer upon polycarbonate support or collagen coated polycarbonate support. They are quite suitable for performing intestinal permeation studies. In order to increase the speed of metabolism studies or to decrease the animal utilization in the metabolism studies, in vitro techniques were developed. Isolated human or animal liver microsomes are incubated along with the drug of interest and at periodical interval the aliquots are subjected for LC-MS or LC-NMR to elucidate the metabolites. Sometimes major metabolites are isolated and subjected to primary in vitro screening to elucidate whether they are active metabolites are not[1].

Several tissue culture methods which measure the rate of chemical absorption by the skin have been approved by the Organization for Economic Cooperation and Development (OECD). The 3T3 Neutral Red Uptake (NRU) Phototoxicity Test, approved by the Organization for Economic Cooperation and Development (OECD), detects the viability of 3T3 cells after exposure to a chemical in the presence or absence of light. Although originally derived from a mouse embryo, the 3T3 cell line was developed in 1962. Neutral red cytotoxicity assay for determining cell toxicity potential, Organotypical skin models for studying irritation of the skin, Hen's Egg Test for mucous membrane compatibility (Hen's Egg Test on the Chorionallantoic Membrane, HET-CAM Test), Photohemolysis test for determining phototoxic potential, Dendritic cells for determining sensitizing potential, The Mouse Local Lymph Node Assay is now accepted by the EPA, OECD, and FDA as the preferred "stand-alone alternative" to the Guinea Pig Sensitization Test.[15], 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[16]. 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, 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[12]. Pyrogens are most often pharmaceutical products or intravenous drugs that may cause inflammation or fever when they interact with immune system cells. This interaction can be quickly and accurately tested in vitro using donated human blood[7].

The MIMIC or modular immune in vitro construct uses human cells to create a model of the human immune system on which the efficacy of new vaccines and other compounds may be tested, replacing some steps of the vaccine development process that would otherwise be performed on animals. This process is faster and more flexible than previous methods but critics worry that it may be too simple to be useful on a large scale[1].

The following alternative methods that can replace legally required tests on animals have been validated and given regulatory approval they are tests for corrosive properties (OECD 430 and 431), tests for acute Phototoxicity or irritation (OECD 432), tests for skin absorption (OECD 428), and in-vitro methods for determining potentially mutagenic effects (OECD 471, 473, 476). The Local Lymph Node Assay (LLNA), which has been approved by the OECD as a test for skin sensitizing properties (OECD 429), makes an important contribution to refinement and reduction. The number of animals needed for certain tests was also reduced by the harmonization of test requirements and the development of new test methods, such as the Acute Toxic Class Method (OECD 423) and the Fixed Dose Method (OECD 420) for testing for acute oral toxicity[16].

The U.S. National Disease Research Interchange provides human tissue to scientists investigating diabetes, cancer, cystic fibrosis, muscular dystrophy, glaucoma, and other human diseases[5].

In vitro genetic research isolated specific markers, genes, and proteins associated with Alzheimer's disease, muscular dystrophy, schizophrenia, and other inherited diseases with tools from molecular biology, biochemistry, and analytical pharmacology. "If you have information on human genes, what's the point of going back to animals?" says Pharmagene cofounder Gorden Baxter[4].

In-silico methods

Substances with similar chemical structures often have similar properties. In these cases, therefore, knowledge of the properties of a few representative substances is sufficient to be able to deduce the properties of a series of similar substances. By analogy, certain properties of these representative substances can also be assumed to be properties of the other substances in the series. The required calculations are performed using specially developed computer programs. It is anticipated that combinations of such calculations will make it possible to narrow down the number of substances to be tested. Only these selected substances will then have to be tested according to the legally prescribed test methods[10].

The last two decades have seen innovations in technology that have helped to evolve automated, microprocessor controlled robotic processes called High Throughput Screening (HTS). This qualitative leap in drug discovery paradigm has been achieved via a synergy of chemistry, biology, engineering and informatics. A similar strategy has also been adopted in studies towards molecular mechanisms of drug action, absorption, metabolism and toxicity studies. In HTS the interactions of ligand with the biological compartment is elucidated by luminescence-based binding assays. Various fluorescence techniques like Fluorescence Anisotropy (FA), Fluorescence Correlation Spectroscopy (FCS), Fluorescence Intensity (FI), Fluorescence Lifetime Imaging Microscopy (FLIM), Fluorescence Resonance Energy Transfer(FRET), Total Internal Reflection Fluorescence(TIRF), and Time Resolved Resonance Anisotropy(TRRA) are used. Along with these techniques, certain specific nano-bead techniques like Scintillation Proximity Assay (SPA), Amplified Luminescence Proximity Homogeneous Assay (ALPHA) are also used[1].

The applicability of computer models has also used completely empirical and statistical models like the Rule of Five or Lipinski's rule. According to this rule, a drug like compound looks like a molecule with a molecular weight less than 500, OH and NH groups less than 5, the sum of N and O atoms less than 10 and log P value less than 5 for a better absorption in the intestine. Computer simulations available include models of asthma, though potential new medicines identified using these techniques are currently still required to be verified in animal and human tests before licensing. Computer operated mannequins, also known as crash test dummies, complete with internal sensors and video, have replaced live animal trauma testing for automobile crash testing. The first of these was "Sierra Sam" built in 1949 by Alderson Research Labs (ARL) Sierra Engineering. These dummies continue to be refined. Prior to this, live pigs were used as test subjects for crash testing[1].

Other non-animal simulators have been developed for military use to mimic battlefield induced traumas. TraumaMan and the Combat Trauma Patient Simulator can be used to simulate hemorrhaging, fractures, amputations and burns. Previously, animals were intentionally subjected to various traumas to provide military training. TraumaMan is also now used for training medical students[7].

Computer models have been constructed to model human metabolism, to study plaque build-up and cardiovascular risk, and to evaluate toxicity of drugs, tasks for which animals are also used[6]. Computer-Aided Molecular Design (CAMD) involves computational analysis of large data set in order to highlight those compounds most likely to be active in the actual assay, so that a focused subset of compounds can be selected. It covers a wide range of technologies leading to very fast property predictions through more computationally elaborate modelling of drug-receptor binding. Using receptor based properties, such as binding affinity and receptor selectivity, CAMD calculates to propose a broad range of properties that are likely to be useful in drug design-from physical properties like molecular size and solubility to indicators of developmental issues like metabolic fate and toxicity etc[1].

Quantitative structure-activity relationships using chemical informatics systems are also used[10].

Ethics

The use of animals in research, teaching and testing is an important ethical and political issue. Much of the discussion about this issue revolves around the relative value, often referred to as 'moral value', of humans and animals. When the needs of animals and humans come into conflict, which takes precedence? Today there exists a wide spectrum of views on this subject, ranging from those concerned with animal 'rights' to those who view animals only as a resource to be exploited. All of these viewpoints have contributed to the development of ethical principles of animal use. These in turn have shaped animal use regulations promulgated by organizations such as Association for assessment and accreditation of laboratory animal care (AAALAC), American association for laboratory animal science (AALAS). These regulations embody principles summarized in statements by the Public Health Service Policy and by NASA. Biblical views of animals are primarily those of utility rather than of moral value, early scholars argued that animals should be treated kindly because animal cruelty represented a flawed morality and was ultimately detrimental to the moral development of humans. This view that humans may ultimately be judged based on their treatment of other lives exists to this day, and for many, is a strong argument for stewardship toward animals[5].

Interestingly, advances in biology that began in the 1800's have provided some of the strongest arguments for imbuing animals with an enhanced moral value. By recognizing that the nervous systems of all vertebrate animals are very similar, it is assumed that activities that will cause a human pain or distress will likewise cause pain or distress to other animals. It is for this reason that current animal use regulations require the use of analgesics, anaesthetics and sedatives for any procedures on animals that may cause more than momentary pain or distress[5, 10].

Animals with advanced nervous systems, such as nonhuman primates, carnivores and marine mammals, have also demonstrated other abilities that humans can relate to and value, such as advanced social behaviour, the ability to react to both positive and negative stimuli, intelligence and even self-awareness. What once had been a clear physical and mental distinction between humans and animals, has become much fuzzier with this new understanding that evolution represents a continuum. Likewise, the assumption that there is a clear moral distinction between humans and animals also has become fuzzier, and it suggests that perhaps gradations in moral value should be applied to animals. This thought is also reflected in modern thinking. Current legislation on animal use emphasizes the idea of replacement of 'higher' animals with 'lower' animals, and requires environmental enrichment or human contact for intelligent, social animals such as nonhuman primates, or dogs and cats, but not for vertebrates like amphibians[5].

Current legislation also recognizes that there are diverse viewpoints about the moral value of animals. Thus, all live animal use in research, teaching or testing must be reviewed by a committee (the IACUC) with diverse membership. There is also an emphasis on minimizing the overall use of animals. Proposals for animal use are reviewed based on the potential for learning new information, or for teaching skills or concepts that cannot be obtained using an alternative. There are also provisions for ensuring that animal use is performed in as humane a manner as possible, minimizing pain, distress or discomfort[7, 10, 12].

An important ethical principle of animal use in biomedical research is that alternatives to live animals should be used whenever possible. There is a legal requirement for documentation of a search for alternatives and an explanation for why these alternatives were not found to be suitable or how alternatives were incorporated into the experimental design[9].

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. 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. As the associate editor of the *British Medical Journal* stated, "When it comes to testing HIV vaccines, only humans will do". Animal experimenters face the unavoidable fact that their artificially created "animal model" can never fully replicate the human condition, whereas clinical investigators know that the results of their work are directly relevant to people[4].

Benefits of non-animal testing

Besides saving countless animal lives, alternatives to animal tests are efficient and reliable. Unlike crude, archaic animal tests, non-animal methods usually take less time to complete, cost only a fraction of what the animal experiments that they replace cost, and are not plagued with species differences that make extrapolation difficult or impossible.

1. Alternative scientific tests are often more reliable than animal tests.

For example, experiments on rats, hamsters, guinea pigs, mice, monkeys, and baboons revealed no link between glass fibres and cancer. Only after human studies related the two, the Occupational Safety and Health Administration (OSHA) label these fibres as carcinogenic. EpiDerm, an *in vitro* test derived from cultured human skin cells, was found to be more accurate in identifying chemical skin irritants than traditional animal tests. In comparison studies, EpiDerm correctly detected all of the test chemicals that irritate human skin, while tests on rabbits misclassified 10 out of 25 test chemicals - a full 40% error rate.

2. The use of human tissue in toxicity testing is more accurate than the animal models.

The "Lethal Dose 50" (LD50) test forces animals to ingest toxic and lethal substances to the endpoint of where 50% of the animals in the study die and those that do not are later killed. The late Dr. Björn Ekwall (Cytotoxicology Laboratory in Sweden) developed a replacement for the LD50 test that measured toxicity at a precision rate of 77-84% accuracy compared to the LD50 rate of 52-60%. This test, far more accurate than the animal models, uses donated human tissue rather than animal. Further, the test can target toxic effects on specific human organs, whether or not the toxic substance permeates the blood barrier, and other highly sophisticated and precise information that the agonizing death of an animal of a different species would not reveal.

3. Non-animal tests are more cost-effective, practical, and expedient.

InVitro International's Corrositex (synthetic skin) can provide a chemical corrosivity determination in as little as 3 minutes to four hours, unlike animal testing that often takes two to four weeks. DakDak, an alternative test used to measure the effectiveness of sunscreens, was reported to do in days what it takes animal studies months to do, and estimates that it can test five or six products for less than half the cost to study a single product in animals. The traditional testing of chemicals using animals can take up to five years per substance and cost millions of dollars, while non-animal alternatives can test hundreds of chemicals in a week for a fraction of the cost.

4. Cruelty-free products are more environmentally friendly.

In toxicity testing, researchers breed, test, and ultimately dispose of millions of animals as pathogenic or hazardous waste. Cruelty-free testing does not damage the environment or create harmful waste[8].

Several Web sites provide descriptions, prices, and ordering information for thousands of alternative learning materials. The following are three excellent databases that focus specifically on alternatives in education they are Humane Society Veterinary Medical Association, InterNICHE, and Norwegian Inventory of Audiovisuals (NORINA) [11].

The following animal protection organizations have established "alternatives loan" programs for students who need to borrow a non-animal software program or other teaching tool in order to satisfy a course requirement so that they will not have to bear the financial burden of purchasing the product they are Ethical Science

Education Coalition, Humane Society of the United States, National Anti-Vivisection Society[9].

Some veterinary schools have also established willed body donation programs. These programs allow clients of veterinary clinics to donate the bodies of their companion animals after they have died a natural death. The cadaver can then be used to train students. Animal cadavers obtained in this way are considered "ethically sourced"[9].

Institutes researching (and organizations funding) alternatives to animal testing include: Centre for Alternatives to Animal Testing, UCDavis Center for Animal Alternatives, Physicians Committee for Responsible Medicine, Dr Hadwen Trust, National Centre for the Replacement, Refinement and Reduction of Animals in Research, Canadian Council on Animal Care Three Rs Microsite, Alternatives to Animal Experimentation Laboratory, Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. In the lab, response of drugs are demonstrated and taught by computer simulation exercises. This is the first such lab in India. In addition, a guide to alternatives to animal experiments in pharmacology is prescribed. Mahatma Gandhi-Doerenkamp Centre for Alternatives to Use of Animals in Life Science Education, India. European Centre for the Validation of Alternative Methods (ECVAM), an online database of toxicology non-animal alternative test methods. Categories at present include *in vitro* methods, QSAR models and a bibliographic section. Under the Framework Programmes 6 and 7, the European Commission is funding a significant number of large integrated research projects aiming to develop alternatives to animal testing[11, 13, 9].

Some resources available to assist in searching for alternatives are listed below. The University of Minnesota Libraries- on-line card catalogs and databases, reference librarians available, The Animal Welfare Information Centre, The Johns Hopkins Centre for Animal Alternatives, The University of California-Davis Centre for Animal Alternatives, The PREX on-line information service, NIH website on Model Organisms for Biomedical Research[7, 11].

CONCLUSION

Research into alternative test methods has so far resulted in the incorporation of a range of new cell and tissue culture systems into the repertoire of alternative methods[8]. Although the efforts in researching alternatives to animal testing methods over the years have produced a number of successful results, a great deal still needs to be done before it will be possible to eliminate animal testing completely. This will require consistent use of the most advanced research methods in the areas of molecular biology and computer technologies[8]. Besides saving countless animal lives, alternatives to animal tests are efficient and reliable. Unlike crude, archaic animal tests, non-animal methods usually take less time to complete, cost only a fraction of what the animal experiments that they replace cost, and are not plagued with species differences that make extrapolation difficult or impossible[4]. Important approaches include the development of in-vitro (in the glass) methods based on biological materials (for example, skin or other human body cells) that will be suitable for reliably verifying the safety and compatibility of product ingredients; the development of in-silico (in the computer) methods to determine the compatibility of substances on the basis of their chemical structure.[10] Human Genome project's first blue print was released on 25th June 2000 and the third map was released in 2001 thereby, throwing light on the hidden biological targets. They need to be elevated for their involvement in various cellular functions and their utilization in various altered physiological conditions. The DNA G-qadruplexes are one of the targets being actively explored for anti-cancer therapy by inhibiting them through small molecules[17]. Nowadays enormous research in the area of gene delivery has been conducted worldwide, in particular for cancer gene[18]. It has been reported that human genome revealed the availability of 750 new GPCRs, 100 ligand gated ion channels, 60 nuclear receptors, 50 cytokines and 20 reuptake/transport proteins. They are all yet to be evaluated for their function.

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