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DIFFERENT APPROACHES TO CONTROL ANIMAL EXPERIMENT

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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 number of animals used in research has increased with the advancement of research and development in medical technology. Every year, millions of experimental animals are used all over the world. The pain, distress and death experienced by the animals during scientific experiments have been a debating issue for a long time. Besides the major concern of ethics, there are few more disadvantages of animal high cost. Various alternatives to animal testing were proposed to overcome the experimentation like requirement of skilled manpower, time consuming protocols and drawbacks associated with animal experiments and avoid the unethical procedures. Different methods and alternative organisms are applied to implement this strategy. These methods provide an alternative means for the drug and chemical testing, up to some levels. A brief account of these alternatives and advantages associated is discussed in this review. An integrated application of these approaches would give an insight into minimum use of animals in scientific experiments.

KEYWORDS: Alternatives, Refinement, In-vitro, In-silico, Animal ethics, Alternative organism, Laboratory animal etc.

Animal is defined as any nonhuman member of the five classes of vertebrates: mammals, birds, reptiles, amphibians and fish. Within this group, two kinds of animals can be distinguished warm-blooded animals (mammals and birds) and cold-blooded animals (reptiles, amphibians and fish). Other creatures customarily included in the animal kingdom,

such as invertebrates (e.g., worms, insects and crustaceans), are excluded by this definition. The use of human subjects is not examined in this assessment.

The concept of alternatives to animal use has come to mean more than merely a one-to-one substitution of non-animal methods for animal techniques. For alternatives, OTA has chosen a definition characterized by the three Rs: replacement, reduction, and refinement.

Scientists may **replace** methods that use animals with those that do not. For example, veterinary students may use a canine cardiopulmonary -resus - citation simulator, Resusci-Dog, instead of living dogs. Cell cultures may replace mice and rats that are fed new products to discover substances poisonous to humans. In addition, using the preceding definition of animal, an invertebrate (e.g., a horseshoe crab) could replace a vertebrate (e.g., a rabbit) in a testing protocol.

Reduction refers to the use of fewer animals. For instance, changing practices allow toxicologists to estimate the lethal dose of a chemical with as few as one-tenth the number of animals used in traditional tests. In biomedical research, longlived animals, such as primates, may be shared, assuming sequential protocols are not deemed inhumane or scientifically conflicting. Designing experimental protocols with appropriate attention to statistical inference can lead to decreases (or to increases) in the numbers of animals used. Or several tissues may be simultaneously taken from a single animal as a result of coordination among investigators. Reduction can also refer to the minimization of any unintentionally duplicative experiments, perhaps through improvements in information resources. Existing procedures may be refined so that animals are subjected to less pain and distress.

Refinements include administration of anesthetics to animals undergoing otherwise painful procedures; administration of tranquilizers for distress; humane destruction prior to recovery from surgical anesthesia; and careful scrutiny of behavioral indices of pain or distress, followed by cessation of the procedure or the use of appropriate analgesics. Refinements also include the enhanced use of noninvasive imaging technologies that allow earlier detection of tumors, organ deterioration, or metabolic changes and the subsequent early euthanasia of test animals. Pain is defined as discomfort resulting from injury or disease, while distress results from pain, anxiety, or fear. Pain may also be psychosomatic, resulting from emotional distress. Although these are subjective phenomena, pain and distress cansometimes be identified and quantified by observing an animal's behavior.[1]

ALTERNATIVES IN RESEARCH

In research, scientists often explore uncharted territory in search of unpredictable events, a process that inherently involves uncertainty, missteps and serendipity. Some biological research requires and in the foresee able future will continue to require-the use of live animals if the study of the complex interactions of the cells, tissues, and organs that make up an organism is to continue. Knowledge thus gained is applied to improving the health and well-being of humans and of animals themselves, and it may lead to the development of methods that would obviate the use of some animals. Some non-animal methods are becoming available in biomedical and behavioral research. As more develop, animal use in research will likely become less common. It is important to note, that even if animals cannot be replaced in certain experiments, researchers can attempt to reduce the number used and also to minimize pain and distress.

Most alternatives to current animal use in research fall into one of four categories:

- **Continued, But Modified, Use of Animals.** This includes alleviation of pain and distress, substitution of cold-blooded for warm-blooded vertebrates, coordination among investigators and use of experimental designs that provide reliable information with fewer animals than were used previously.
- **Living Systems.** These include micro-organisms, invertebrates and the in vitro culture of organs, tissues and cells.
- **Nonliving Systems.** These include epidemiologic databases and chemical and physical systems that mimic biological functions.
- **Computer Programs.** These simulate biological functions and interactions.

The many fields of research ranging from anatomy to zoology use animals differently and each thus has different prospects for developing and implementing alternatives. To determine the prevalence of animal and non-animal methods in varied disciplines of research, OTA surveyed 6000 articles published between 1980 and 1983 in 12 biomedical research journals and 3 behavioral research journals. Research disciplines were distinguished by their characteristic patterns of animal use, as measured by the percentages of published reports showing animal use, no animal use and use of humans. Animal methods predominated in most of the journals surveyed, including the three behavioral research journals. The exceptions in the overall survey were cell biology, which used primarily non-animal methods, and cardiology, which used primarily human subjects.[1]

Using alternative methods in biomedical research holds several advantages from scientific, economic, and humane perspectives, including

- reduction in the number of animals used;
- reduction in animal pain, distress and experimental insult;
- reduction in investigator-induced, artifactual physiological phenomena;
- savings in time, with the benefit of obtaining results more quickly;
- the ability to perform replicative protocols on a routine basis;
- reduction in the cost of research; greater flexibility to alter conditions and variables of the experimental protocol;
- reduction of error stemming from interindividual variability; and
- the intrinsic potential of in vitro techniques to study cellular and molecular mechanisms.

Many of these alternative methods are accompanied by inherent disadvantages, including

- reduced ability to study organismal growth processes;
- reduced ability to study cells, tissues and organ systems acting in concert;
- reduced ability to study integrated biochemical and metabolic pathways;
- reduced ability to study behavior;
- reduced ability to study the recovery of damaged tissue;
- reduced ability to stud-y interaction between the organism and its environment;
- reduced ability to study idiosyncratic or species-specific responses;
- reduced ability to distinguish between maleand female-specific phenomena; and a handicap to probing the unknown and phenomena not yet identified.

Behavior encompasses all the movements and sensations by which living things interact with both the living and nonliving components of their environment. Since one of the chief goals of behavioral research is an understanding of human behavior, there are obvious advantages to the use of human research subjects. There are also advantages to using animals, including the following:

- Laboratory research on animals offers a greater opportunity to control variables such as genetic background, prior experience, and environmental conditions, all of which affect behavior and can obscure the influence of the factor under study.
- The short lifespans of certain animals allow scientists to study behavior as it develops with age and across generations.

- Some animal behavior is less complex than human behavior, facilitating an understanding of basic elements and principles of behavior.
- The behavior of certain animals holds particular interest for humans. These animals include companion species, farm animals and agricultural pests.

Although behavior is a biological phenomenon, behavioral research differs substantially from biomedical research in that researchers have fewer opportunities to study mechanisms isolated from living organisms. There is little prospect, for example, of using in vitro cultures to look at aggression, habitat and food selection, exploration patterns, or body maintenance activities—all topics studied by behavioral scientists. Yet in each of these disciplines, reduction or refinements of animal use may be possible. It is the continued, but modified, use of animals that holds the most promise as an alternative in the field of behavioral research.[1]

ALTERNATIVES IN TESTING

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. 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. 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. **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. 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. 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 test10. In August 2010, OECD has published the Test Guideline 439 which describes the new procedure for in vitro hazard identification of irritant chemicals 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.

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 birthdefect testing in rats and rabbits. 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.

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. 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. 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. 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 isolated specific markers, genes and proteins associated with

Alzheimer's disease, muscular dystrophy, schizophrenia, and other inherited diseases with tools frommolecular biology, biochemistry, and analytical pharmacology.[2]

ALTERNATIVES IN EDUCATION

Although far fewer animals are used in education than in either research or testing, animal use in the classroom plays an important role in shaping societal attitudes toward this subject. As educational goals vary from level to level, so does the use of animals and therefore the potential for alternatives.

In elementary schools, live animals are generally present solely for observation and to acquaint students with the care and handling of different species. Although the guidelines set by many school boards and science teachers' associations limit the use of living vertebrates to procedures that neither cause pain or distress nor interfere with the animals' health, these guidelines are not observed in all secondary schools. Science fairs are an additional avenue for students to pursue original research. The Westinghouse Science Fair prohibits the invasive use of live vertebrates, whereas the International Science and Engineering Fair has no such prohibition.

In the college classroom and teaching laboratory, alternatives are being developed and implemented because they sometimes offer learning advantages, are cheaper than animal methods and satisfy animal welfare concerns.

As a student advances, animal use at the post-secondary level becomes increasingly tied to research and skill acquisition. As graduate education merges with laboratory research and training, animal use becomes largely a function of the questions under investigation. In disciplines such as surgical training in the health professions, some measure of animal use can be helpful but is not universally viewed as essential.

Many alternative methods in education are already accepted practice. Replacements include computer simulations of physiological phenomena and pharmacologic reactions, cell culture studies, human and animal cadavers and audiovisual materials. Clinical observation and instruction can also replace the use of animals in some laboratory exercises in medical and veteri- nary schools. Reduction techniques include the use of classroom demonstrations in place of individual students' animal surgery and multiple use of each animal, although subjecting an animal to multiple recovery procedures may be viewed as inhumane and

counter to refined use. Refinements include the use of analgesics, euthanasia prior to recovery from surgery, observation of intact animals in the classroom or in their natural habitats, and the substitution of cold-blooded for warmblooded vertebrates in laboratory exercises. Humane education aspires to instill positive attitudes toward life and respect for living animals. Instruction in proper care and handling of various species may be complemented by exposure to the principles of animal use in research and testing and to alternative methods. This type of education promotes attitudes conducive to the development and adoption of alternatives.[3]

COMPUTER MODELS

Computers can help to understand the various basic principles of biology. Specialized computer models and software programs help to design new medicines. Computer generated simulations are used to predict the various possible 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 predict 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. Hence, the total number of experimental animals is lowered and the objectives of Russel and Burche's 3 Rs are achieved. Another popular tool is the Structure Activity Relationship (SARs) computer programs. It predicts biological activity of a drug candidate based on the presence of chemical moieties attached 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 activit. The activities like carcinogenicity and mutagenicity of a potential drug candidate 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.[4]

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 important alternative 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. *In vitro* culture of animal/human cells includes their isolation from each other and growing as a monolayer over the surface of culture plates/flasks. Cellular components like membrane fragments, cellular enzymes can also be used. Various types of cultures like cell culture, callus culture, tissue culture and organ culture are used for various purposes. Benefits associated with techniques are, easy to follow, less time consuming and are less expensive. These methodologies are routinely used for preliminary screening of potential drug molecules/chemicals to check their toxicity and efficac. Almost all cosmetics, drugs and chemicals are tested for their toxicity and efficacy, using these tests. For example, eye irritancy test. To check the irritancy of chemicals previously Draize test was used, which requires animals (mainly rabbit). It is very painful and every time a new animal is used. Ke Ping Xu and coworkers suggested an alternative which uses bovine corneal organ culture. The bovine cornea is cultured up to three weeks in laboratory and various analytical methods are used to evaluate the toxicological effect of test chemical irritancy *in vitro*.

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.

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.[5]

ETHICAL CONSIDERATIONS

At one end of a broad spectrum of ethical concerns about animal use is the belief that humans may use animals in any way they wish, without regard for the animals suffering. At the other extreme is the notion--epitomized by the slogan ‘(animals are people, too’’ —that each animal has the right not to be used for any purpose that does not benefit it. Each view is anchored in a school of philosophical thought, and people considering this issue can choose from a variety of arguable positions.

Prominent within the Western philosophic and religious tradition is the view that humans have the right to use animals for the benefit of humankind. This view is predicated on the assumption that human beings have special intrinsic value and thus may use natural animate and inanimate objects, including animals, for purposes that will enhance the quality of human life. Yet this tradition suggests that because animals are intelligent and sentient beings, they should be treated in a humane manner. Current policies and trends within the scientific community have reinforced this conviction by advocating that pain and suffering be minimized when animals are used in research, testing, or education.

Advocates of what generally is called animal welfare frequently question the objectives of animal use, as well as the means. They point out that animals can experience pain, distress, and pleasure. Drawing on the utilitarian doctrine of providing the greatest good for the greatest number, some animal welfare advocates weigh animal interests against human interests. In this view, it might be permissible to use animals in research to find a cure for a fatal human disease, but it would be unjust to subject animals to pain to develop a product with purely cosmetic value.

Some animal rights advocates carry this concern a step further and do not balance human and animal rights. They generally invoke the principle of inalienable individual rights. They believe that animal use is unjustified unless it has the potential to benefit the particular animal being used. Animal rights advocates refer to the denial of animal rights as a form of "speciesism," a moral breach analogous to racism or sexism. Animals, by this reasoning, have a right not to be exploited by people.

People throughout the spectrum find common ground in the principle of humane treatment, but they fail to agree on how this principle should be applied. Society does not apply the principle of humane treatment equally to all animals. A cat may evoke more sympathy than a frog, for example, because the cat is a companion species and possesses apparently greater neurological sophistication than a frog, endowing it with both favored status and a familiarity that suggests to humans that they can interpret its behavior. Even within a species, all individuals are not treated consistently. Pet rabbits in the home and pest rabbits in the garden, like human friends and strangers, are treated differently.[6]

The improvements in public health and safety made possible through the use of animals in research and testing are well known. But these questions remain" Do these advances justify animal use? How much of the improvements were actually dependent on the use of animals? Debate on these and other questions is bound to continue, but most parties agree that consideration of replacing, reducing and refining the use of animals is desirable.

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.

In Vitro 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.[7]

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. 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. 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. 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. Nowadays enormous research in the area of gene delivery has been conducted worldwide, in particular for cancer gene. 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.[8]

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