Use of Animals

in

Scientific Research

May 2000

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1. Introduction

From time immemorial, man has depended on animals for his survival, either as food (cattle, sheep, pigs, poultry etc.) or for competition and companionship (horse, dog, cat, parrots etc.). As he knew more about his surroundings, he extended this dependence to acquisition of knowledge, dating back to the days of the great physician Galen (129-200 AD), who used animals to demonstrate that arteries contained blood and not air. We have come a long way since then and specially bred laboratory animals consisting of mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, monkeys, higher farm animals and a variety of birds and other lower forms are now integral part of biomedical research.

2. Why are animals used in experiments ?

"Research , in reality, involves three facets : acquisition of new knowledge, use of animals in teaching exercises, and the testing of compounds, chemicals or devices for safety and effectiveness. There must be reasonable expectation that research involving animals will contribute significantly to present and future knowledge, which may eventually lead to the protection and improvement of the health and welfare of either humans or animals. World over, new drug research as well as tests meant for assuring the quality and efficacy of pharmaceutical products /vaccines/biological are based on experiments involving animals. Toxicological studies specially those performed in rodents and beagle dogs are the essential link between the pre clinical phase and clinical development of the drug molecule. No new drug can be introduced in clinical practice or even for the matters into clinical research unless it passes the battery of toxicity tests in animals.

Human biology is very much like that of many other animals. That is why, results from animal experiments apply to people. Most laboratory animals have the same set of organs - heart, lungs, liver, and so on which work in the same way as they do in humane. There are seven major areas of medicine and biology where animals for experiments need to be used.

i. ***Fundamental biological and medical research*** - This is necessarily to be undertaken as to unravel the secrets of nature. If we essentially know how different tissues and organs are kept healthy, we can then find out what goes wrong when disease strikes. Fundamental research in biology and medicine are basic foundations on which future discoveries are based.

ii. ***Developing new treatments for diseases*** - To conquer disease, a lot of work needs to be put into, by way of developing better medicines, perfecting surgical operations as well as making vaccines and finding other ways of preventing diseases. Even though

much of this work is for humans, many of them are applicable to animals as well, and even some are exclusively for animals. There are many diseases which are yet to have a proper cure like multiple sclerosis, certain cancers, as well as new diseases like AIDS, Alzheimer disease etc. All these need initial input in terms of animal experiments.

iii. ***Preparations of natural products used in medical research and treatment*** - Animals can produce useful medical substances in their blood or milk, like antibodies, vaccines and hormones which are important for diagnostic tests, medical treatments, and basic research. May be at a later date, we can produce all these synthetically, but at present, most of them are produced using animals.

iv. ***Safety testing of chemicals and drugs*** - A wide range of chemicals and medicines which are used in day-to-day life, as household products, in farming, industry etc., need to be tested for their safe use in humans as well as in animals. Such preliminary testing is very much essential for avoiding pollution and associated health hazards and proper healthy maintenance of the environment.

v. ***Study of genetic disorders*** - There are many diseases which are inherited fully or partially and are caused by basic faults in a person's genetic code. Some of the animals also have similar genetic fault as humans do. There are mutant strains like dystrophic mice which have the same faulty gene as the muscular dystrophic patients. The animal thus plays a vital role in understanding and treatment of such genetic diseases. Scientists have now made such progress in molecular biology that they can now alter genes and breed strains of mice and other animals with particular genetic diseases. This may ultimately lead to treatments in genetic disorders like cystic fibrosis, sickle cell anaemia and other diseases which run in families.

vi. ***Development of new diagnostic tests for diseases*** - If the treatment of a disease is to be effective, an accurate and quick diagnosis is essential. Animal experiments are vital in this area, which include scanning of unborn babies for identifying cancers, diagnose heart diseases etc. Animal tests have paved the way for many blood tests for the diagnosis of infectious diseases.

vii. ***In biology and medical education*** - The animals are to be used in teaching biology in schools and colleges in understanding the basic anatomy and physiology of man and other animals.

Medical Research in the last 100 years has facilitated many ways to treat and prevent diseases in humans as well as in animals. Table 1 lists some of the main discoveries in basic research related to animals experiments. The development of many major medical treatments has also depended on animal research. Table 2 lists some of the well known examples of these.

TABLE 1

MAJOR ADVANCES IN BASIC RESEARCH THAT DEPENDED ON ANIMAL EXPERIMENTS

1600's	-	Discovery of blood circulation Discovery of the function of the lungs
1700's	-	Measurement of blood pressure
1800's	-	Vaccination to stimulate immunity Understanding of infectious diseases
1900's	-	Discovery of antibodies Understanding of hormone systems
1920's	-	Discovery of vitamins
1930's	-	Discovery of the mechanism of nerve impulses Discovery of tumour viruses
1940's	-	Understanding of embryonic development
1950's	-	Understanding of the control of muscle activity Understanding of energy metabolism Understanding the mechanism of hearing
1960's	-	Discovery of monoclonal antibodies Understanding the biochemical functions of the liver
1970's	-	Understanding of transplantation antigens Understanding the way the brain functions Discovery of prostaglandins
1980's	-	Development of transgenic animals Understanding the basis of memory
1990's	-	Understanding auto-immune disorders *In vitro* fertilization, cloning, gene manipulation

TABLE 2

MAJOR MEDICAL ADVANCES THAT DEPENDED ON ANIMAL RESEARCH

1920's -	Insulin for diabetes
1930's -	Modern anaesthetics for surgery
	Diphtheria vaccine
1940's -	Broad-spectrum antibiotics for infections
	Whooping cough vaccine
	Heart-lung machine for open-heart surgery
1950's -	Kidney transplants
	Cardiac pacemakers and replacement heart valves
	Polio vaccine
	Drugs for high blood pressure
	Hip replacement surgery
1960's -	Corneal transplants
	Rubella vaccine
	Coronary bypass operations
	Heart transplants
	Drugs to treat mental illness
1970's -	Drugs to treat ulcers
	Improved sutures and other surgical techniques
	Drugs to treat asthma
	Drugs to treat leukaemia
1980's -	Immunosuppressant drugs for organ transplants
	CAT scanning for improved diagnosis
	Life-support systems for premature babies
	Drugs to treat viral disease
1990's -	Laproscopic surgical techniques
	Breast Cancer Links
	Gene therapy for cystic fibrosis

3. Opposition to animal use for research

In spite of the advancement in biomedical research, and the benefits derived by the society through them, the opposition to animal experiments always existed. It has a long history, dating almost from the day animals were being used in biomedical research. From writing to peaceful protests in earlier days, the movement has become more violent in recent times, especially in Europe and America.

The animal right movement which originated in Europe and America does not seem to be due to any religious believes. The Bible, for example, is unequivocal about the position of animals in the natural order: *`God made man in his image and gave him dominion over all other creatures'*. On the other hand, oriental and particularly Indian attitude to animals is one that is tinged with religious and ethical colours. Hinduism and Buddhism envisage a hierarchy of organisms rather than a sharp division. The Hindu pantheon places many forms of plants, birds and animals on par with humans and deifies them. Animals and birds are thought not only as Vahanas or Vehicles on which God rides, but much more useful as well. Over the centuries this has brought about a very healthy respect in the Indian mind for all forms of life. The cow is sacred not because it is a divine vehicle alone, but because it has an overall utility value. Buddhism and Jainism carries this attitude further, leading to vegetarianism and respects for all living beings. To the Sufis, steeped in equally considerate attitudes the prevalent Indian mind set was extremely acceptable. Thus, in the east, regardless of specific sectar religions, the attitude to other life forms was not exploitative, but appreciative. Even pigs, boars, buffalos and monkeys are referred in holy books and the Indian mind set can become easily sensitive when it comes to these animals. These religious sentiments could be one major reason why the animal activism in this country has found firm roots, while in the West it may be because of the writings of some secular philosophers. The foremost in this group was, Jeremy Bentham an English Barrister who in 1780 questioned the lack of moral regard of humans to fellow animals. In 1970's people like Singer, Dian Fossay and Jam Goodall echoed these sentiments further especially with regard to use of primates. But the actual animal rights movement took roots from the uncompromising ideas of Tom Regan who wrote the famous `The case for animals rights' (1983). Regan believed that all humans and most animals have inherent rights, and that their bodies cannot be transgressed, no matter how much good might there by result. He took the extreme view that animals cannot be experimented on, because they are not really means to an end.

The central dogma of antivivisection movement laid down by Regan believes that the `end do not justify the means'. It takes a simplest view of animal experiments arguing `that the means causing the animals to suffer, is not justified by the ends namely the increase in biological knowledge or the relief of human suffering'. On this basis, the activist feels that all experimental procedures which cause animal to distress should be discontinued. This view is supported by two arguments; firstly, that the animals have similar rights to humans and that experimenting on them without their consent is indefensible as it would be with people.

Secondly, antivivisectionist draws attention to the poor quality of science on animals and some of them maintain that animal experiments have not led to significant advances in human and animal medicine. They also contend that pure scientific enquiry cannot be justified because it is simply a question of curiosity.

According to animal activists, any animal research project implies a potential violation of six widely subscribed moral norms and values. These are the following: respect to animals as they are, perform good science, be a good citizen, have responsibility for future generations, have responsibility for environment and show respect for life style and religious orientation of people. Under the first category, the breeding of animals under caged conditions, feeding them prepared diets, handling them for experiments and sacrificing their lives are violations of biological species specific life. Inducing pain, causing suffering, anxiety, stress or impairing their psychological well being are abuses which deviate even more from the norm of reverence to life. Induction of heritable deviations of the wild type genome-transgenic animals, genetically modified organisms, present the latest class of violation against the normal norm. In addition to aspects of behavioural and genetic naturalness, aesthetic naturalness also appear to be important. For example, a mouse bearing a grafted human ear is repulsive to many. The nude mouse, a research animal well accepted within the subculture of molecular biologists, also triggers a strong gut response in lay public. The animal identification methods practised for laboratory animals like clipping, notching ears, fingers, toes and cutting of tissues from the tail to check the transgenesis of new born mice amount to deliberate mutations in the eyes of animal lovers.

According to antivivisectionists, toxicity testing in animal do not confirm to good science practice. This is the most controversial of all animal procedures because many people believe that it is unnecessary to test many of the compounds. Although not all animals used in toxicity suffer (eg control or low dose groups), a significant number do, because the intention of toxicity testing is to induce adverse effects in some of the animals. These tests are intended to identify which organs of the body, may be susceptible to damage by a particular chemical. Many such toxicity tests normally uses more animals than necessary to reach a valid conclusion, overuse statistics to claim significance (LD_{50} for instance),use statistics without any related biological theory (for example - mutagenic risk assessment), unravel molecular process *in vivo*, when *in vitro* would have given better control of relevant variables.

Further, many commercial laboratories, and contract research do not reveal the results of many of the procedures used, leading to unnecessary duplication of experiments inadvertently. Requirement for protocol research formulated by international bodies (like World Health Organization, Organizations for Economic Co-operation and Development) still lag far behind in recent insights into assessing the risk for consumer. LD_{50}, Draize test(application of test chemical into the eye of Rabbit to check for irritation) are still followed in many countries as a statutory requirement of safety evaluation of chemicals, cosmetics and other consumer

products.

To the animal lover, to be a good citizen implies respect to your neighbours and pets, be tolerant, honest and modest and do deeds which set good example to your children, family and society. Unfortunately, in the eyes of antivivisectionist animal experimentalist do not fall under this category. In the West, and in many affluent societies in other parts of the world, cats, dogs and other animals are kept as pets and it is unthinkable for them to do any experiments on such animals. Even the laboratory rodents and rabbits are kept by many as pets, and to many animal lovers, experiments on these innocent dumb creatures amounts to bad citizenship.

Animal moralists feel that scientists are irresponsible towards future generations. Even though all biomedical research is aimed at innovations and/or improvement of therapies against human or animal disease, many feel that large amount of wealth is being squandered away, in the name of science, which would have been otherwise useful for future generations. They especially criticise high-tech medical therapies which benefit only a few rich in private hospitals, and they feel that more money should be spend for general public health care and gerontology. In recent times, development of transgenic animals for xenotransplantation has been viewed as a great risk of transferring disease to future generation rather than the gain of temporary well being of some thousands of individual patients. The activists blame the scientists for not putting enough effort in developing hi-tech alternatives to animals, which they feel are the responsibility of scientists for future generation.

4. Responsibility of Scientists

Experimental animals must be managed and cared for through the application of uniform acceptable standards, in facilities designed for animal holding, in caging which provides for comfort and safety. As well the animal's social and behavioural need must be addressed : Unnecessary pain, stress, and anxiety should be avoided, but may inadvertently be caused by inadequate caging or animal facilities, or improper handling by personnel.

Humane painless handling of animals, humane treatment of all experimental animals including proper maintenance of living conditions and minimisation of distress constitutes a hall mark ethic of a civil and democratic society.

The scientists should be deeply concerned about the rational and humane use of animals in research. Ethics committee should be functional in institutions. They should be concerned about avoiding unnecessary pain or suffering or injury to animals during holding, experimentation and post –experimentation period by monitoring and improving their housing, environment, feeding and veterinary care. This can be achieved by providing accredition services to laboratories by constituting, National Accreditation Board of Testing and Calibration Laboratories (NABL) having membership of the International Laboratory

Accreditation Cooperation. This such accreditation of animal facilities would demonstrate their commitment to responsible animal care and use and good science since such an accreditation is an indicator of an institution's ability to comply with its assurances.

Animals should be used only if the scientist's best efforts to find a replacement technique have failed. A basic tenet of any scientific study using animal should be the adherence to the "3Rs" tenet of replacement, reduction and refinement. This should be linked to a continuing review of the literature and a sharing of information methodology and experience.

Animals free of clinically recognizable or latent disease, and of appropriate genetic stock of strain should be chosen. They may be obtained from an inhouse production system, from commercial companies, or possibly farmers and other hobbyists having an interest in breeding and raising animals. Whoever the supplier, they must understand the importance of genetic quality and breeding techniques, recognise disease and maintain healthy stocks of animals. It is important that such individuals recognize that "dirty animals produce dirty science".

The working objective and ethical requirement for the use of animals in research dictates the need to use the best methods, on the smallest number of appropriate animals required, in order to obtain valid scientific information. Proposed experiments must be justifiable in terms of the declared objectives. The experimental design must offer every practicable safeguard to the animal. Animals lives should not be wasted because of inadequate knowledge of the requirements of the species being used, incorrect management or handling techniques, inappropriate or incorrect experimental design, or lack of surgical knowledge and experience. It must be remembered that animals can and do suffer if knowledge, practices, and proper procedures are lacking. Animal care is everyone's responsibility.

In the use of animals in research, it must be recognized that contrary to human experimentation, consent can never be given; therefore, the animal knows no reason nor sees any benefit that may be derived from its use. Although man and other animals are known to be beneficiaries of research, it is the animal on experimental study which experiences the pain and the results of the procedures, and does not know when the pain might end or be relieved. Pain, if it exists, is something the animal experiences now, and to it, now is forever.

Worldwide, there is consensus that the deliberate cruelty to animals is repugnant, and must be avoided. If meaningful scientific results are to be accrued through the use of animals in research, teaching and testing, the scientific community in every country must ensure humane and ethical treatment of animals is achievable and worthy of consideration before the use of animals is permitted.

5. Ethical arguments

By adopting an extreme moralistic standard, the animal activists look at animal researchers as cruel and corrupt, consumed by desire for ever more papers and grants. The fundamental pragmatic value of biomedical enquiry to both humans and other animals is the relief of human and animal suffering and the enhancement of opportunities for individual activity and well being'. `If possible, relieve suffering', is the fundamental motto of all biomedical researchers, and relief of human suffering is the basic aim of all biomedical enquiry, without belittling the animals used in the process. The scientists involved in biomedical research have a duty to perform and the Institutions are maintained by public funds for this purpose.

Enquiry, is a fundamental capacity and activity, characteristic of human life, in which we are not the only ones who might have a stake. We are an integral part of animal's nature and seemingly are life's most emphatic, if not the only, opportunity to know, understand and explicitly appreciate itself. Curiosity arises from our organic depths, and the thrill of a novel idea or discovery infuses our whole bodily being. Science is an ongoing adventure, requiring both an intensely individual and co-operative efforts. It has an inherently civilizing force, bringing people together for humanely noble reasons, in a mixture of co-operation and competition that seems to mark a vital high civilization. This appears on local, national, international levels and involves more than the scientists, all those who directly or indirectly participate in a scientific culture. Given the ethical imperatives of scientific enquiry and the integral role of animals in biomedicine, there seems a prima facie justification for the scientific use of animals both for the practical benefits to human and animal life. Another fundamental principle in nature, is that all forms of animals including human life must by nature use other instances of organic life in order to be alive and flourish. This precarious worldly interaction and capture of energy is a fundamental law of metabolic existence. Man and animals have coexisted in nature and many animals have been domesticated and used by man for work, entertainment as well as for food. Scientific pursuits using laboratory animals go into higher realms where work pursued through them is for improving the quality of life both for humans as well as for animals. The laboratory animals which are tamed over the years and kept under controlled conditions cannot be released into wild any more, since by the same law of nature they may fall easy pray to the predators lurking in the dark. This goes against the very moral principle, the activists preach to the society and does more harm to the animals whom they wish to protect. The production of animal models, in terms of natural mutants, or transgenics, as long as they are for pursuing a higher scientific goal need not be denied to the scientists.

However, several ethical dilemmas arising from the very nature and limitations of science are inescapable. For example, it has been well argued by animal researchers that animal experimentation leads to important theoretical and practical results, benefiting both humans and animals. But at the same time it cannot be denied that many experiments lead nowhere, despite animal suffering and loss of life. There is difficulty in judging prospectively which particular experiments will lead to significant results and which will prove useless. Even with calculations of probability of success, it is difficult ethically to face this not so productive use

and harming of animals. There are also factors of serendipity, luck or chance in biomedical enquiry, which defy rational calculations.

An area in which scientists usually face ethical dilemma is the toxicity testing on animals. Toxicity tests are routinely employed in laboratories around the world for testing new chemicals (intermediates in industrial process and chemical ingredients used in cosmetics) and new commercial products (pharmaceuticals and pesticides). These tests are intended to identify which organs of the body may be susceptible to damage by a particular chemical. Such tests are usually required for statutory regulations designed to protect health welfare of both workers/manufacturers and consumers and they generally aim at establishing the degree to which new chemical compounds have potentially toxic or irritant effects. Also all drugs intended for clinical use should undergo toxicity testing in a rodent, carnivore and a primate before it is taken for human trials. It may be much easy to justify the drugs for treating AIDS, than testing the safety of the latest shoe polish or lipstick. But the latter cannot be avoided; since the same society uses it for its aesthetic enhancement and this is an industry driven predicament. Unless there is change in the mindset of the people using these products, such trivial tests need to go on along with the ethical conflict it produces in the minds of the users as well as testers.

Another moral dilemma which the scientists face is when they have to use higher mammals more close to them like cats, dogs and primates. To pursue a particular scientific goal, the scientists should have the same dispassionate consideration for all the models they use, be it a mouse or monkey. But it is much easier said than done. In fact, many of the animal welfare movements have its roots in the handling of these higher forms by scientists. To many, cats and dogs are more than a companion and the use of these and primates evoke a sense of empathy even to scientists. But if special ethical consideration has to be given to the use of the higher forms then it would halt potentially significant basic or applied research. With new diseases in the horizon, like AIDS and bovine subacute spingiform encephalopathy (BSE), in which both the humans and the higher mammals are interlinked, such sentiments have little value. But the dilemma persists because of the similarity and closeness of the test system.

The advent of biotechnology with its capacity to alter the nature of life has placed scientists into an unenvious position of heartless explorers who have no consideration for their immediate environment, and the people who live in them. They are even considered irresponsible towards future generations. But we are in an exciting era, where many genetic diseases, which earlier thought to be unconquerable appear to be with in our grasp. Should not this knowledge be harnessed, so that benefits from it will flow to both humans and animals equally. As long as there are proper containments, controls and regulation in the area of genetic manipulation, the benefits to be derived from such technology should be appreciated by the public. Unfortunately, there is lack of proper communication between scientists and the public, especially in this controversial area, resulting in half truths and mislaid notions. Thus

the transgenics, knockouts and Dolleys have made the scientific tribe more unpopular than before. They are in the midst of exciting and explosive research, and with commercial interest looming large for such new products, scientists do not know where to start and where to end, since they do not call the shots any more. Commercial exploitation of newer discoveries adds to the already existing predicament.

6. The balancing act

The lay public often get confused by the rigid postures assumed by the animal activists on one hand and the animal researchers on the other. After all, the general public is the beneficiary for whose benefit the war is fought by both the sides. A proper balancing seems to be necessary, where each side understand and appreciate the view point of the other. The animal activists contend that animals have rights just as human being do, and animal experimentation is a violation of these rights. Sigma xi, a scientific honour society in USA made a useful distinction between animal rights and animal welfare. In terms of ethics they argued that the term right carries with it concomitant responsibilities. In other words, rights and responsibilities always go hand in hand. Human beings have the rights, and thus they have a responsibility towards non-human beings surrounding them. No responsibilities are attributed to non-human beings and intrinsically, they cannot have the same right as enjoyed by human beings. Once the rights are understood, the scientists should not view the animals as mere machines, and the earlier Judean-Christian attitude of treating all animals as having been placed on earth for man's use should be abandoned. Darwinism clearly shows that all animal life is related through evolutionary process and some species such as apes are sibling species to humans. We are clearly related to chimpanzees as tigers to cats and horses to donkeys. The theory of evolution teaches further that animals have feelings, are capable of suffering and experiencing pain and pleasure, because we share much of our inherited emotional make up with them. Thus, the people who uses animals for research and other purposes should keep them well and treat them with respect. They should be used only for legitimate purposes and with in the limitations of the experiment. Every reasonable effort should be made to minimise pain or discomfort. The non-human animals have a right to enjoy these welfare measures, and the researches who have a right of different kind, are bound by strong responsibility to provide these measures. The animal rights movement then should be changed to an animal welfare movement and the animal researchers should equally reciprocate to these concerns. It is heartening to see that such a change has already come into existence in Europe and America, which also gave birth to the extreme antivivisectionist. Universities Federation for Animal Welfare (UFAW) based in UK, and the Animal Welfare Institute and Hastings Center based in USA are some such centers which work with professionals to improve the wellbeing of all animals. These organizations seek to persuade those responsible for animals to provide the best conditions possible for their living, with in limitations imposed on them, and with in the framework of existing animal laws. India too is on the verge of establishing the National Institute of Animal Welfare.

7. Animal welfare measures - the responsible experimentation

Once the experimenters acknowledge the right of animals to co-exist with dignity, responsibility, the obligations squarely falls on the former's shoulders. The first and foremost of this is the continued commitment to the welfare of animals under their care. Not only should we care for them but we should also be seen to do so. Usually one can recognize two main sources of suffering by laboratory animals: husbandry conditions, including the effect of restricted space, diet, social isolation or unusual social grouping and environmental conditions including noise and smell of other animals; and scientific procedures involved in experimental research like handling, oral dosing, injections, withdrawal of body fluids, withdrawal of food, withdrawal of specific nutrients etc. The improvement of husbandry conditions can be effected by knowing the basic requirements of animals in terms of space, movement, food, social - interactions and alleviating the suffering due to scientific procedures and through professional training in humane techniques aimed at minimising pain and distress in animals. On a large scale this can be achieved by minimizing the use of animals in scientific research, testing and education by resorting to the three `R' principles of Replacement, Reduction and Refinement advocated by British Zoologist William M.S. Russel and the microbiologist Rex L. Burch in 1959. Although it took few decades to catch on the three R's currently define the search for viable alternatives to, if not fully, but partially to animal experimentation.

8. Need for Guidelines

Every country which uses animals for research has come out with a set guidelines for the care and use of laboratory animals, which include housing, feeding and humane caring. The guidelines are uniform throughout the world, and strict adherence to these guidelines are made compulsory by appropriate animal welfare laws. These guidelines insist on standard environmental conditions to be provided to the animals in terms of light, temperature, humidity, ventilation, noise levels etc. and also the standard nutritive diet to be provided to them. Traditionally laboratory animals are kept in barren and boring cages. But in recent years there has been a move towards enriching the surroundings of these animals within the bounds of experimental requirements. Environmental enrichment involves increasing the complexity of the cage so that animal finds it more stimulating and it is encouraged and able to behave more naturally. Enrichment can take a variety of different forms depending on the animal. It can for example, involve toys, bedding and nesting materials, novel feeding techniques or social interactions. This is more true for higher mammals like cat, dog and primates. Unlike rodents they should not be caged singly for long periods, and for their well being they should have the freedom of moving periodically into larger areas (runs) enriched with stimulating objects for exploration and group interactions.

National bodies are set up in many countries for the proper care and use of experimental animals and there is an International Committee for Laboratory Animal Science (ICLAS)

(currently the office of the President of ICLAS, based in USA), with a membership of about 100 countries. The ICLAS has taken up the job of setting up international guidelines for animal husbandry, experimental procedures, teaching and training of researchers and professionals in the field. In India such guidelines were published by Indian National Science Academy (INSA) in 1992 and recently revised in 2000.

9. Provision of professional training

The most important scientific goals and well designed protocols are worthless, if those who handle the animals are improperly trained or unskilled. Professional bungling and inadequacy of animal care with ensuing animal distress can skew experimental data and render a particular experiment useless. Attention to the training of personal concerned with animals upkeep, and the researchers who use these animals should be the prime concern of any institution using animals.

Laboratory animal science is a nascent science which mainly encompasses the fields of biosciences, medicine and veterinary and other disciplines. Its basic aim is the know-how of animals used in research (mostly rodents, rabbits, cats, dogs, primates and a variety of other species) and the appropriate way to handle them for various experimental procedures. It is an advanced area of specialization, and nowhere this science is taught at the degree level even in the veterinary curriculum. In Europe and America one to two year diploma course run by laboratory animal bodies are available for biology, medical and veterinary graduates. In India and other developing countries national bodies concerned with laboratory animal sciences conduct short term training courses ranging from 6 to 12 weeks for those who are involved in the husbandry of the animals and to those who use them. The latter category of people are more important, because their exposure to animals are for a short time when they are using animals. In many developed countries, every scientist starting research on animals has been required to take a three week orientation course. They have to learn hands on procedures, proper anaesthesia, specification of inbred strains and so on, as well as the three R's. Assessment of pain and distress to animals during upkeep and experimental procedures is an integral part of this training, and the trainees have to be familiar with physiological and behavioural signs of pain, control and alleviation of pain through environmental and behavioral measures and through drugs. They need to be familiar even with euthanasia procedures when and wherever they are applicable. In India National Centre for Laboratory Animal Sciences (NCLAS) at Hyderabad under the ICMR and Central Drug Research Institute (CDRI), Lucknow under the Council for Scientific and Industrial Research (CSIR) administer such training programmes regularly.

10. Welfare measures through legislation

Countries all over the world especially the developed countries, have strict legislations

controlling experiments using animals. These animal laws cover a wide spectrum starting from housing, record keeping, scientific procedures - involving handling, oral feeding, injections, withdrawal of body fluids, pain perception, surgery, euthanasia etc.- and training of personnel working in the field. The earliest to enact such a law was the UK government in the year 1876 followed by all European countries, North America and Asia oceano Pacific countries in 1960's. There are certain common features in most of the laws, but there is certain amount of dichotomy between the individual countries' laws in its definition and interpretation. In general, a centralised body at the national level and a local ethical institutional committee at the research Institute level is central to all these laws. The laws are also very specific emphasizing the need to have trained manpower in the field. But at the interpretation and implementation level there are yawning gaps from country to country. In UK, Australia, Germany and several other nations cost benefit analysis is to be performed before an animal experiment can proceed. The Netherlands Government gives equal right to animal and man, whereas it is not so in other European and American nations. Accreditation schemes based on housing, and the availability of trained professionals are common in all developed countries and special home inspectors, check the premises periodically to assess the standard of the facilities. Further, a valid licence is essential for the laboratories performing animal experiments, and even for scientists to hold animals for experimental purposes and to perform experiments on them. In the educational field countries like UK and Netherlands have banned practising animal surgery in medical schools, whereas in USA the ban is limited to few states. The penalty clause for non-compliance of rule vary from fines to revoking of licence to perform experiments. In India, animal dissection is banned till high school level but made optional at the Senior Secondary School level through Gazette notification recently.

The laws are generally silent on the rehabilitation of animals used in experimental work. While the rodents are generally culled, this is not usually advocated in higher mammals especially in case of primates. These animals are often reused for other investigations, wherever possible. But their rehabilitation afterwards has not been spelt out properly by policy makers as well as by enforcement authorities.

The legislation in developing and poor nations are not as stringent as in the developed countries. This may be due to lack of awareness and proper communication and also because of the inability, to meet the standards set up by the law, on account of financial restraints. There is thus a genuine need to harmonise all the existing animal laws in the world. But it should be remembered that countries other than developed nations, need more time and money to catch up with the former and strict enforcement of animal laws at this juncture without creating proper atmosphere will only jeopardise the progress of ongoing work in these countries.

11. Issues in Animal Experimentation

The ultimate goal of any animal welfare measure is the elimination of all experiments on animals that are likely to cause pain or distress. But this can be only be a dream, at present since, new and new disease(s) keep propping up and sometimes eliminating one may provide for another one to flourish. So the next best measure is to think of strategies that can eliminate the use of higher mammals altogether or at least reduce the number as much as possible. Such conscientious measure(s) was started in good earnest by the introduction of 3 `R' principles by Russel and Birch in 1953. The importance of their approach lies in its combination of animal welfare considerations along with good science and best practices. They defined the R's in the following way: reduction as a means of `lowering the number of animals used to obtain information of a given amount and precision', 'refinement' as any development leading to a `decrease in the incidence of severity of inhumane procedures applied to those animals, which have to be used' and 'replacement' as any scientific method `employing non-sentient material which may replace methods which use conscious living vertebrates'.

Refinement encompass all those methods described above (good housing, professionally qualified keepers and users) and include use of new techniques developed in human medicine aimed at decreased invasiveness of bodies, improved instrumentation for monitoring animals and analysis of body fluids and better management of pain and distress in animals. A sophisticated instrument like magnetic resonance imaging can save the life of many animals which are otherwise to be sacrificed at timely interval, to understand the time course of a drug action or a basic process. The use of such sophistication requires the use of a single animal to provide all information along a given curve. With the advent of microelectronics, fibre optics and laser technology, animals can be monitored externally and internally without restricting their movement in their primary enclosures. A variety of non-experimental variables associated with prolonged restraint, thus can be removed, contributory to defined and reliable data. Another improvement in instrumentation is the development of diagnostic clinical laboratory equipment, which requires only microlitter sample to perform a variety of diagnostic tests. The use of smaller sample size means the use of smaller, animal species and further it prevents the need to euthanise many of these species to obtain the required volume of blood. It is now possible to obtain serial blood samples from small laboratory rodents which reduces the number of animals necessary to obtain data over the length of the study.

The aim of any **reduction** strategy is for obtaining comparable levels of information from minimum number of animals or obtain more information from a given number of animals, so that towards the end, a given project or test is completed with few animals than originally contemplated. Such reduction(s) can be attempted on several ways like animal sharing, changes in research strategy,use of proper statistical design, proper use of strains, mutants, transgenics etc. Animal sharing refers to an internal system adopted by any laboratory wherein whenever the animal(s) is sacrificed at the end of the experiment, the organ, tissues or body fluids not needed for that project can be shared amongst other researchers. Culling of morbid or very old animals (especially rodents) is a normal practice in many animal facilities. The

organs and body fluids from such animals can be frozen and stored and later made available to researchers whenever they require these materials for standardising certain biochemical tests/procedures. Both these measures eliminate unwanted killings and can bring about substantial reduction in the use of animals in absolute numbers. Research strategies adopted should be such that reduction in number is the primary goal. For example before starting a major experiment involving large number of animals, small pilot studies using few animals could be tried out to see the trend. In toxicity studies non-mammals (insects, marine invertebrates, microorganisms, plants) or *in vitro* or test tube methods or even non-living systems (chemical or physical systems like computer simulation) can be tried as pre-screen strategies. There is enough studies to suggest that poor experimental design, together with inappropriate statistical analysis of experimental results leading to inefficient use of animals. So proper statistical design, prior to undertaking any study and appropriate analysis of the resultant data, can give comparable or greater precision using fewer animals. Coming to type of animals to be used, it should be remembered that a heterogenous or out bred group of animals lead to large amount of variation causing non-reproducible false data and the necessity to repeat the experiment in many cases. Inbred strains, specific mutants, transgenic and knock out animals are made to order assembly products designed to give precise and uniform results in an experiment.

Replacement is the ultimate in animal welfare measures, where given purpose is achieved without conducting experiments or other scientific procedures on protected live animals. The replacement can be absolute or relative, direct or indirect, total or partial. When a humane killing of vertebrate animal is done to provide cells, tissues and/or organs for the *in vitro* studies, it is a relative replacement, to live animals. But if permanent human and invertebrate cell and tissue cultures are maintained for toxicity screening, that becomes absolute replacement. If a tissue (say skin) from human or dead animal is used *in vitro*, instead of the same tissue from live animals, it amounts to direct replacement. Replacement can be indirect as in cases where pyrogen test in rabbit is replaced by Limulus amoebocyte lysate (LAL) or by a test based on whole human blood. Total avoidance of an animal procedure because of lack of justification or reliability of the method, using a human volunteer instead of a laboratory animal, or testing a chemical in vitro instead of live animals - all tend amount to total replacement. But if on the contrary, non-animal methods are used as pre-screening in toxicity studies, so that most toxic compounds can be rejected before continuing with further tests in animals, one has achieved a level of partial replacement. Based on the various terms of replacements described above, different approaches can be practised by researchers. Some of the important strategies are as follows:

a) Avoid unnecessary repetition of experiments, and use alternatives where ever possible by seeking access to on-line data base, which give up to date lists of published research in all areas of science.

b) Use immunochemical systems to replace bioassays for detecting bacterial toxins, study

enzyme structures and mechanisms of action or computer linked mannequin for teaching basic principles of medicine and applied techniques.

c) Use mathematical and computer modelling like molecular modelling (for drug design, and structure-activity relationship of drug versus, biological activity site), physiologically based pharmacokinetic modelling (prediction of disposition of xenobiotics and their metabolites by integrating species-specific physiological parameters, partition co-efficient of the chemicals and metabolic parameters) and specified computer programs to study anatomy, physiology and other processes for education and training purposes (compu-rat, compu-frog, programmes for rat and frog biology).

d) Use *in vitro* methods, which include sub-cellular fractions, tissue-slices, cell suspensions and perfused organs, and tissue culture proper (cell and organotypic cultures), including human tissue culture, in toxicity testing and for preliminary screening.

e) Use lower organisms with limited sentience/ or not protected by legislation controlling animal experiments which include invertebrates (Drosophila, the nematode - Caenorhabditis elegens, the earth worm, coelenterate - Hydra attenuate - the horse shoe crab - Limulus polyphemus etc.) plants, fungus, bacteria (Salmonella) and viruses. Some of them are useful as pre-screen system, especially for agrochemicals and environmental pollutants, and also for genotoxic, studies and endotoxin detection.

f) Use early developmental stages of vertebrates before they reach a point at which their use in experiments and other scientific procedures is regulated. For example, by US laws, the early stage is considered half away through gestation in mammals, or incubation in birds and reptiles at the stage where independent feeding occurs as in amphibians and fishes. For example embryos of xenopus, chicken and mammalians whole embryo cultures are ideal for studying early detection of reproductive toxicity, teratogenicity and for elucidating mechanisms of teratogenesis.

g) Use human tissues and volunteers, wherever possible to avoid the problem of inter specific extrapolation from animals to humans and to get more mechanistic information. Some of the examples are use of immortalised human cell lines like keratinocytes for drug studies, and the use of foetal and placental tissues. In nutrition studies, and some times for dermal toxicology, human volunteers can be sought.

Starting from 1960 most of the developed countries in Europe, North America and Japan have put in considerable amounts for providing **alternative strategies** to animal research. Among several countries, In the world Germany and Netherlands are in the forefront in these

approaches and an European Center for validation of alternatives was set up recently by European Commission in the US the government interest is comparatively low; but the alternative center established at John Hopkins University do get funds for grant disbursal from government as well as from industries. The efforts put in by these centers have yielded some good results in terms of reduction of animal use. For example the classical LD_{50} (used in toxicological studies) which normally require a minimum of 200 rats can now be done with anywhere between 3 and 18, because of statistical sophistry. In the LD_{80} test for vaccine the end point is no longer the death of 80% control animals but simply a check in the rise in the level of antibodies. The German center for alternatives found out that sensitivity of chemicals need to be tested only in mice and rats, skipping dogs altogether. Production of polio-vaccine is a recent success story. In 1970s in Netherlands as many as 5000 monkeys were required for this purpose; but now kidney cell cultures from just 10 monkeys seem to be more than adequate to cover the entire population. Recently transgenic mice having a human gene was found to be very effective in testing polio-vaccine. In reality, the hormones and vaccines manufactured in cell cultures are purer than those made in vivo, so each batch need not be tested as before for safety and efficacy. The corrosive skin test done on a rabbit's shaved back for chemicals is now replaced in USA with restructured human skin or by a biomembrane called Corrositex.

The cosmetic manufacturers in these countries have entirely eliminated animal testing; relying on in-house substitutes or use ingredients that have been tested in the past. In India, testing of cosmetics on animals for batch to batch variation or use of tested chemicals has been made optional to the manufacturers recently. There is also a world wide trend to use more and more herbal cosmetics, which use natural products from plants eliminating the need for animal tests altogether. However, in general, replacement procedures are only for applied research. For basic and fundamental studies reduction and refinement are the only alternative(s) available.

12. Special needs of developing countries

Developing countries are striving to achieve the professional standards set up by the developed nations, but in this venture they are severely handicapped by resource crunch, as the available finances are primarily diverted towards human welfare. At the same time many of the new technologies developed in the latter are needed to improve the health standards of humans and animals in this part of the world. One way to achieve this goal is by technology transfer and liberal financing by the developed countries. In this new era of world trade based on patent regime, this is easier said than done. On one hand there are genuine concerns in those countries about the exploitation of their diversified fauna and flora by the developed nations. But on the other hand, this cannot be avoided unless indigenous cheaper technologies are developed. Previously, in 70's large number of rhesus monkeys were imported to USA and European countries. But looking at the crass and crude ways they were used in some of the studies abroad, a ban was affected subsequently in 80's. Some of the animals present in this

part of the world may have unique biology suitable for a particular research need and it is quite possible that these animals are imported across borders quietly. This can cause depletion of the existing stock and moreover the country itself is deprived of its genuine use.

Biomedical research involves highly specialized techniques often available in only few laboratories. Globally research organisations utilize these by getting the studies done there are paying for them. The strategy has several advantages. Reliable data can be generated at these centres of excellence using minimal number of animals unnecessary duplication of facilities and wastage of animals is avoided and the specialised centre gets fund to maintain and improve upon the expertise. Such arrangements should be facilitated by local governments.

There are certain species of animals needed for specialised studies which are difficult or uneconomic to breed. For eg. Filarial infection in longoor monkey produces disease similar to the human disease. Leprosy is best produced in armadillo. Similarly large number of mongrel dogs and cats are available and are needed for work on cardiovascular system or on vomiting, which can not be produced in rodents. Limited trapping of such animals, rhesus monkey etc in approved number by licensed vendors and from approved locations has to be permitted.

In view of global competitiveness, there is an urgent as well as strong need to synthesise molecules as new chemical entitles which may be considered for IPR protections provided data on these entities can be generated in specific genetically engineered strains, species and animal models for disease like diabetes, hyperlipidaemia, hypertension, immunodeficiency and cancer etc. It becomes crucial for the laboratories to develop facilities where these activities are thoroughly evaluated and labs are able to provide data which is acceptable to regulatory authorities in USA as well as in European Community. Unless we able to get these opportunities within the Country, it would be extremely difficult for the Scientists as well as institutions to obtain global marketing rights for drugs. Hence, import of animals has to be liberalised in such special instances.

In developing countries there is a great need for the strengthening of research resource, in view of prevalence of various infections diseases both emerging re-emerging. There are great numbers of people suffering from diseases still lacking effective cure. These diseases are rare or absent in the developed countries which have their own health and other problems requiring the research efforts for their solution. Thus developing countries must carry out effective research on their own problems.

In the years to come there will be a need for more and better laboratory animal houses in developing countries because of an increased demand for biomedical reseach, sera, vaccines and drugs. The developing countries have specific needs for cheaper drugs, and vaccines to protect their people and animals from existing infectious diseases and the newly evolving disease like AIDS, BSE etc. With strict enforcement of animal welfare laws prevailing in

developed countries, the animal testing can now be totally conducted within the developing countries.

For many purposes animals of good health status will be required, and for breeding it may be necessary to maintain animals at a higher health status i.e. specific-pathogen-free. Good health will be promoted by ensuring that animals receive a satisfactory diet and have adequate housing and that they receive care from competent staff.

Most developing countries have their own socio-economic problems unique to their geographical location and their historical, political and educational backgrounds. The problems may lead to very little considerations for laboratory animals by all concerned. The attitudes towards animals and animal experiments may be very different. Most established laboratory animal units have been initiated by foreign assistance, and soon after aid was withdrawn the standard of animal care and management declined, or the facilities went out of use because running costs could not be paid. Sufficient funding must be granted to meet basic capital and recurrent costs of animal units.

Traditional and religious backgrounds may lead to certain problems if there is no proper trainig given to animal caretakers and users. In some social cultures, it is not the practice for qualified staff to carry out duties involving animal handling or care, but these inhibitions must be overcome before a laboratory animal unit can operate efficiently and animal welfare is ensured.

Due to constraints in finances, any availability of specialized strains of animals (mutants, transgenics), and alternative technologies of drug testing is still beyond the reach of these countries. Till such time the situation improves, these countries need to import all these animals from abroad. And since these strains have special requirements for their maintenance, provisions also should be there for infrastructure development and trained professionals. Same is true for developing alternate technologies to animal experimentation. The developing nations have the required manpower; what they need is proper training and access to modern tools (animals, instrumentation) of biomedical research. The developed countries have the technology and the money, and they are in the look out for newer animal models and cheaper labour. Once proper areas are identified which are of importance to both the sides, bilateral agreement can be made protecting the interest of involved nations. For this to emerge there should be harmonization of the existing rules prevalent in different countries and both should have commitment to undertake projects which are of universal appeal to humans as well as animals and the benefits should be shared in an equitable manner.

13. The scenario in India

The scenario in India, with respect to laboratory animals is far from satisfactory. Even though we made a head start as early as 1960 along with other countries, our progress has been tardy due to various reasons. Among the scientific organisations, the Indian Council of Medical Research (ICMR) realised the importance of laboratory animals and a unit called Laboratory Animal Information Service (LAIS) was started at the Indian Cancer Research Centre now called (Cancer Research Institute, Mumbai). This unit for the first time undertook a survey on the conditions of animal facilities in the country and in two years started regular courses at the junior level for animal handling and experimentation. This unit subsequently got shifted to National Institute of Nutrition, Hyderabad, wherein in 1986 it received additional support from Dept. of Biotechnology, and developed into a National Centre for Laboratory Animal Sciences (NCLAS). The centre can now boast of genuine inbred strains of mice and rats and other species, which are maintained in conventional and microbiologically and genetically defined status. It has recently developed mutant strains of rats for the study of obesity and diabetes which has some specific traits unlike the models available abroad. The Centre has also developed animal diets in pellet form using locally available ingredients. It caters to the breeding and experimental needs of over 180 institutes in this country and it has regular training programme at the junior and senior level for animal house personnel. The Central Drug Research Institute, Lucknow, is another national Centre in India where the emphasis is on primates as well as on alternate technologies. Apart from these two centres there are about 10-12 good animal facilities in different parts of the country at the public and private sectors. But these animal facilities are all of the conventional type.

There are over 400-500 animal facilities in the country, spreading over research institutions, public and private pharmaceutical companies, universities, medical and veterinary collages. Many of the animal facilities especially the ones attached to universities, medical and veterinary collages have no proper infrastructures in terms of building, housing etc. Proper record keeping and quality control measures are totally lacking in many of the facilities. Many of the facilities do not have well defined inbred strains and the country is yet to develop its own specialised mutants, transgenics and knock out animals, that would have improved the quality of the work and also reduce the number of animals used in research. Yet, compared to Western countries our intake of laboratory animals is very low, approximately around one tenth of the number used by them. Many animal facilities do not have a separate animal budget. The existing inferior situation is mostly due to lack of enough funds rather than lack of awareness of good laboratory animal husbandry practices.

India enacted an animal law as early as 1960's called the `Prevention of Cruelty to Animals Act' amended in 1982 currently (undergoing revision) which provided for the prevention of cruelty to animals in general. Under Chapter 4 of the same Act there is provision for the control of experimentation on animals. The act also provides the constitution of an Animal welfare board to take care of the welfare of the animals in general, and also provides that the Animal Welfare Board constitute a **Committee for the Control and Supervision of**

Experiments on Animals. This Committee is empowered to take care of the legal and ethical aspects of experimental animals being used in research and enact preventive measures wherever there is violation of the law. The CPCSEA had initially appointed home inspectors who visited various facilities in the country and reported the status of the facilities. But their reports for improvement of the facilities were not abided by many, and even the inspections stopped subsequently. In recent times, the CPCSEA has become more active and it is now made mandatory that every animal facility in the country be registered and the facilities have a local Institutional Ethical Committee to monitor the experimental work going on in their respective institutes. The CPCSEA issued a Gazette Notification in December 98 called "Breeding of and experiment on Animals (Control) and (Supervision) Rules, 1998" and its amended notification in February 2001 to accomplish the above intent which are being debated for further refinement.

There is a distinct lack of trained manpower, at the senior level in many of the facilities. Laboratory Animal Sciences is an emerging new field and it is not covered in the curriculum of any of graduate courses in the country be it veterinary, medical, pharmaceutical or any biosciences course. Some of the veterinary courses in the country have allocated some meagre hours for such an important field and the syllabi is also very superficial. In the medical curriculum the subject is not covered at all and in pharmaceutical and biological courses, though practical classes involving the anatomy and physiology of animals are included, there is not much coverage on the care, breeding and management aspects. It should be made compulsory, that every person working with the animals either at the management level or for experimentation should have a certificate in laboratory animal care and procedures. It can be of short duration for research workers who need to orient specifically for the experimental procedures which they may likely to use in the course of their work. But those who are managing the facilities, need to have a longer training (6 months to one year).

Another area of concern is the use of large animals like cats, dogs and monkeys for experimental work. Unlike Western countries, there are very few facilities in the country, where controlled captive breeding is undertaken for these animals. With the longer duration of gestation, and weaning and limited litter size (especially in monkeys) in these animals, enough animals cannot be raised by these limited facilities for many of the experimental needs of the country.

Looking at the inadequacy of many of the animal facilities in the country, it is advisable to have at least 6-8 good National Centres wherein genuine strains of animals are maintained including mutants, transgenics and knock out animals. A corpus fund contributed by the Ministry of Health, Ministry of Science & Technology, Ministry of Commerce & Industry and Ministry of Environment & Forests should be established to ensure sustained funding to keep these Centres in the most modern way meeting higher standards. They can take up basic and experimental work and can also advise and monitor small experimental facilities at

other research institutes, university departments and even the private and public pharmaceutical companies. This way they could help to develop small facilities needing the required standards.

Development of alternate strategies to animal experimentation can be part of the objective of the National Centres or a separate Institute for alternate research methodology and validation can be established. Any alternate me [22] which has been validated for its reliability and reproducibility should be then incorporated replacing animal experimentation in that particular area.

Since it is imperative that some selected studies and safety evaluation of certain drugs need to use cats, dogs, primates and other large animals, a programme for their captive breeding have to be initiated. This is very much true for primates and there is a need to establish 2 or 3 national centres of excellence to breed primates in captivity and to undertake work related to reproductive biology, disease problems, behavioural status and population dynamics. We should try to preserve and facilitate the growth of a sustained populations of different species of primates unique to our country. These centres should be large enough to meet the genuine needs of the work involving the use of primates.

Animal right movement in the country is in the early stage of anti vivisection as witnessed in Europe and America in early 60's. There are a number of private organisation in the field, the prominent ones being Blue cross, PETA, SPCA etc. For the large interest of human and animal betterment in the country, there appears to be an urgent need for the activists and the scientists to sit across the table and iron out the differences in their individual perceptions. This will help to channelise the current animal right movement in the country into an animal welfare movement and pave way for the genuine scientific research to flourish for the benefit of humans and animals.

14. Conclusion

There are several broad fields of influence that impact on shaping public attitude and public policy. They stem from academia, the legislature, the courts and from the individual agendas of the protagonist parties. In the matter of animals issues they range from introduction of animal issues into undergraduate curriculum, courses on animal ethics in veterinary schools and development of alternatives. It must be emphasised that use of animals in research is inevitable and cannot be abandoned in the interest of human and animal welfare. Animal experimentation is necessary at the current level of knowledge for studying the pathogenesis of different disease, undertake drug trials, generate a variety of biologicals such as immuno-diagnostics, vaccines to alleviate suffering in the human and animals themselves. In vitro alternate methods cannot replace animal experimentation totally but can work only as adjuncts and reduce the number of animals to the extent possible. This is why the use of animals continue to be mandatory to meet the statutory requirements. However, efforts to develop

alternate methods should continuously be made so that the day will be reached when no more animals are used for experimentation.

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