

# Use of animals in research

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*There have been some recent reports questioning the use of animals in drug research. The propriety and value of using animals in medical and drug research has been the subject of much public debate for decades in Europe and USA; in UK a Royal Commission was appointed in 1875 to examine this issue. Over the years a consensus has emerged that the long-term benefits to humans and animals which result from the discovery of new drugs and acquisition of new knowledge requiring the use of animals outweigh the consideration of causing suffering to animals. The eradication of small pox since mid 1970s, the control of rinderpest disease in cattle, one of the deadliest diseases of live-stock and the likely eradication of polio, leprosy and filaria in humans in the first quarter of the next millennium are some of the dramatic illustrations of the benefits brought to us by drug research. The use of animals in biomedical research is thus considered an unavoidable necessity; it is a balance between suffering entailed by animals and the long-term gains from new drugs and new knowledge. However, there has been a continuous refinement in the approach to the use of animals both to reduce the numbers and also to make the experimental conditions as humane as possible. Guidelines have been drawn up for animal experimentation by international and national bodies, and form an essential part of the Good Laboratory Practices (GLP) which are recommended to be followed. The pressing need for new drug discovery research in India, the scientific and regulatory compulsions for the use of animals therefore, and the ethical and GLP guidelines available for animal experimentation are examined in this article, and some suggestions made regarding the regulation of the use of animals in research.*

## The need for new drug discovery research in India

THE need for India to undertake new drug discovery research is very pressing. With the great disparities in the life-styles in our population there is a very wide spectrum of diseases prevalent. There are a large number of people, perhaps equal to those in the whole of Europe, who suffer from diseases which are common in developed countries. Then of course we have our special needs: drugs for fertility control and for parasitic diseases such as malaria, filaria and leishmaniasis. No multinational company would be interested in developing drugs for these conditions as there is no market for these drugs in developed countries where 80% of the drugs are consumed; as the cost of new drug discovery research escalates, the multinational pharmaceutical companies (MNCs), who today are responsible for discovering/developing most of the new drugs, are becoming more selective to develop drugs only for those diseases which would have large markets. And if we do not develop

drugs for our special needs who else would! This becomes all the more important in the context of our membership of WTO; from 2005, when we enter the era of the new patent regime, our access to new drugs through process patents will be cut off. We, therefore, have to develop new drugs not only for our special needs, but also for other diseases which are common in the affluent countries like cardiovascular and metabolic diseases and cancers. The latter areas will become specially important as our pharmaceutical companies become global and must have new drugs to market. We have some special strengths to exploit in this endeavour. We have a large resource of new drug discovery leads in the materia-medica of our traditional systems of medicine which we could exploit to our benefit. Then of course new drug discovery research (NDDR), especially clinical research, costs much less in India. Though exact figures would be difficult to compute, at a rough estimate it would be 1/5 to 1/10 times the cost in developed countries. It is therefore imperative that we take up new drug discovery and development research in a big way. It is heartening to note that a number of our forward-looking pharmaceutical companies have set up in-house NDDR Laboratories. We should not take any step that will put back the clock of new drug discovery research in India.

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## Drug regulatory laws

One important function of the Drug Control Administration is to ensure that new drugs registered and licensed for marketing are safe to use. The Drug Controller General of India (DCGI) has prescribed rules and regulations specifying the type of toxicity and safety studies which have to be carried out. Expert committees examine all the preclinical toxicity data, based on which the DCGI gives permission for studying the efficacy and safety of new compounds in humans. The human trials include Phase I (safety) and Phases II & III (clinical evaluation studies). While each country would lay down its own set of regulatory laws, there is a great deal of consultation and harmonization between different countries in this regard as the drugs discovered would be used by different countries throughout the world. The current regulatory laws in-force in India describing the number of animals to be used are available in *Safety of Toxicology Studies for Drug Development*, 1994 published by DCGI. Table 1 is reproduced from this document which specifies the types and numbers of animals to be used for preclinical toxicity studies.

Use of animals, including dogs, in new drug discovery and development studies is thus a mandatory/statutory regulatory requirement laid down by the DCGI, in harmonization with regulatory requirements of many other countries. No new drug introduction can take place without conforming to these regulatory requirements.

## Ethics of animal research

The present outcry and gory evocative images created on the use of animals in research are nothing new; such outbursts have been there in other countries too – except that this happened in those countries many decades ago, which was followed by discussion of the various issues involved. Gradually, a balanced view emerged on the cost-benefit analysis – the cost is mainly the animal suffering inflicted, while the benefits include the addition of new drugs to save lives and alleviate human and animal suffering and acquisition of new knowledge. A lot of attention has been given to assessing these factors

and also on finding ways of minimizing the discomfort and suffering caused to animals and to optimize the gains of knowledge from the minimal use of animals. A synoptic and balanced articulation of these concerns is available in the book, *The Principles of Humane Experimental Technique*<sup>4</sup>. This book in essence has defined the following three Rs which should determine the approach and goals in the use of animals in research: (a) replacement of animals by *in vitro*, or test-tube methods, wherever possible; (b) reduction in the number of animals by means of refined statistical techniques, without losing the significance of numbers; (c) refinement of the experimental methods so as to cause minimal suffering to animals and avoid unnecessary repetitions.

Paton in his book *Man & Mouse* published in 1984 while discussing the balance to be drawn between the benefits reaching into the future and the suffering entailed added a fourth R, 'responsibility', to be shared between the scientist to conduct 'good science', design experiments properly, so as to get definite answers and not waste animals, and on the public to support this work for the 'larger good' that it may lead to.

The three (or four) Rs have since been sharpened by many bodies, such as the 'European Centre for the Validation of Alternative Methods', a body set up in 1992. In essence their concern has been to reduce the numbers of animals to be used for any investigation. As a result, the controversy regarding the propriety of the use of animals in research has declined considerably and it is more or less agreed that a certain number of animals (and normal human volunteers) have to be used in new drug development research. However, the concern for humane use of animals remains high. The 'animal activists' should not feel that it is only their prerogative to have concern for animal welfare. Many animal experimenters are also animal lovers and many of them, I am sure took to drug research as a mission to reduce suffering both of humans and animals. It is a practice among many scientists who consider life in any form with sanctity, to say a little prayer before any animal experiment – that the experiment is being done to add to the pool of knowledge and to promote the process of 'life', thus showing their 'reverence for life'. This in fact should be the fifth R, which should regulate the

Table 1. Number of animals for long term toxicity studies

Group	2-6 Weeks				7-25 Weeks			
	Rodents (Rats)		Non-rodents (Dogs)		Rodents (Rats)		Non-Rodents (Dogs)	
	M	F	M	F	M	F	M	F
Control	6-10	6-10	2-3	2-3	15-30	15-30	2-6	4-6
Low dose	6-10	6-10	2-3	2-3	15-30	15-30	2-6	4-6
Intermediate dose	6-10	6-10	2-3	2-3	15-30	15-30	2-6	4-6
High dose	6-10	6-10	2-3	2-3	15-30	15-30	2-6	4-6

use of animals in research. So 'animal activists' should not abrogate only to themselves the responsibility of safety of animals.

An illustration of the kind of changes that have taken place in the use of animals over the years, the approach defined in the R principles is given below. In the classical LD50 acute toxicity (at a dose causing 50% of animals to die) studies, up to 200 rats and 20 dogs or other animals were employed. With statistical sophistry, almost one-tenth the number of animals are at present considered essential and are used. The Organization for Economic Cooperation and Development (OECD) asks for between 3 to 18 animals data—if the substance kills the first three, it needs to be tested no further. Similarly, in skin sensitivity test for cosmetic safety testing, the rabbit skin test has been replaced by reconstructed human skin or biomembranes. Similar reduction in the use of animals has and is taking place in vaccine production. For polio vaccine production, where earlier large number of monkeys were used, kidney cell cultures are now used which require a much smaller number of animals. So, the present balanced view is that the animals cannot be totally replaced in biomedical and drug research, but efforts to reduce the numbers being used, going down in phylogenetic tree and refinement in experimental methods to make them as humane as possible should be continued.

### The scientific basis of animal testing

The biological testing of products, whether for specific physiological/pharmacological activity or for general toxicity/safety is based on some broad general considerations. These include:

- Essentially similar biochemical and molecular processes characterize all biological systems/living forms.
- However, as we move up the evolutionary ladder and as hierarchy of living forms rises (from unicellular to multicellular to invertebrates to vertebrates and finally humans) there is generation of greater complexity with increase in structure-function segregation giving the higher forms evolutionary survival benefits. This has made each organism special and unique and the closer the organisms are in evolutionary development, the more similar they would be in physiological responses; but, no two organisms would be totally identical. As in final reckoning, no animal will show exactly the same response as a human being, all that is done is to reduce the burden of scientific investigation as far as possible by first testing in animals, which simulate as closely as possible the required human response.

In view of these considerations, biological testing for

products meant for use in humans follows the following phylogenetic hierarchy:

- *In vitro* systems: Isolated tissues or receptors
- Microbial systems
- Rodents: rats, mice, guinea-pigs
- Dogs and/or monkeys
- Humans

These test systems are constantly reviewed. The main guiding principles are the three Rs described above to rely as far as possible on *in vitro* and microbial systems to reduce the number of animals, and to make the experimentation on animals as humane as possible. In the present state of knowledge, it is not possible to totally replace the use of animals or humans in drug discovery research/biomedical research.

The story behind the discovery and introduction of sulphonamides, which ushered in the era of chemotherapy, in fact of modern drug research, illustrates in a striking manner the importance of the use of animals in drug research. The team investigating these compounds led by Gerhard Domagk at Bayers Laboratories in Germany, found no antibacterial activity of the sulphonamides in *in vitro* tests (on bacteria grown on agar plates), but decided to test some dyes containing a sulfonamide residue such as prontosil for their antibacterial activity in infected mice (using the so-called mouse-protection test) and the compounds were found to be very active, even though they had no effect on bacteria grown on agar plates; the active antibacterial sulfanilamide was formed from prontosil within the body by metabolic breakdown. Then followed the discovery of more powerful antibacterial sulfonamides and they were used successfully against many infections. The impact of this discovery was tremendous; for the first time, drugs became available which had direct effect on the bacteria and deaths from bacterial infections (both in humans and in animals) dwindled sharply. Domagk won the Nobel Prize in 1939 for his work on antibacterial sulphonamides. Many lives would have been lost if Domagk had not used animals for his experiments!

In the case of penicillin, the practical application of its use as an effective drug (after Fleming's discovery in 1928) was delayed by almost a decade because Fleming did not use an animal model to prove the efficacy.

Another concern is not to disturb the natural *biodiversity* and *ecological balance*. The experimental animals should be primarily laboratory bred for experimentation. This would also ensure the quality and pedigree of the animal, which are important considerations to get dependable experimental data.

### Guidelines

Broad guidelines have been drawn up and are available for GLP in human and animal experimentation. In 1985

the Council for International Organizations of Medical Sciences (CIOMS) published guiding principles for biomedical research involving animals. Based on these and other such discussions each country has evolved its own guidelines and practices. In India, the Indian National Science Academy, New Delhi has drawn up the guidelines for the use of animals in research as summarized below:

1. Animal experiments should be undertaken only after due consideration to their relevance for human or animal health and the advancement of knowledge.
2. Animals selected for an experiment should be of an appropriate species and quality, and minimum number should be used to obtain scientifically and statistically valid results.
3. Investigators and other personnel should treat animals with kindness and should take proper care by avoiding or minimizing discomfort, distress or pain.
4. Investigators should assume that all procedures which would cause pain in human beings may cause pain in other vertebrate species also (although more needs to be known about the perception of pain in animals).
5. Procedures that may cause more than momentary pain or distress should be performed with appropriate sedation, analgesia or anaesthesia in accordance with accepted veterinary practice. Surgical or other painful procedures should not be performed on unanaesthetized animals.
6. At the end of or when appropriate during an experiment the animal that would otherwise suffer severe or chronic pain, distress, discomfort, or disablement that cannot be relieved or repaired should be painlessly killed under anaesthesia.
7. The best possible living condition should be provided to animals used for research purpose. Normally the care of animals should be under the supervision of a veterinarian or a person having adequate experience in laboratory animal care.
8. It is the responsibility of the investigator to ensure that personnel conducting experiment on animals possess appropriate qualifications or experience for conducting the required procedures. Adequate opportunities have to be provided by the institution for inservice training for scientific and technical staff in this respect.
9. *In vitro* systems, to replace or reduce the number of animals, should be used wherever possible.

Each research institution/laboratory using animals is supposed to have an Animals Ethics Committee to regulate, supervise and monitor the use of animals in research following these guidelines. The Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) under the Ministry of Environment & Forests should ensure that these guidelines are implemented and followed.

The scientists using animals should recognize that 'animal activists' have focussed their attention to con-

stantly review measures which would promote animal welfare, which they might not otherwise have considered. Thus, the two sides must appreciate each other and do what will add to our pool of knowledge and also promote the humane use of animals. Banning experiments on animals on the premise that there are scientifically and morally superior alternatives, is scientifically untenable. The scientists using animals and the animal activists must work together to appreciate each other's point of view better. We must get over the syndromes of 'we' and 'they', and change it to 'we together'. It would be useful for Institutional Animal Ethics Committees to have animal activists as members and the animal activists organizations to have working scientists as their members.

### Present scene in India for animal-based research

From the above consideration it is clear that for the right balance between benefits reaching into the future and the suffering to the animals that may be entailed, animal experimentation has to be continued for new drug discovery and biomedical research. Guidelines for care and use of animals in biomedical research have been drawn up by the Indian National Science Academy. There is a provision for each laboratory using animals in research to have an Animal Ethics Committee to regulate and supervise the experiments on animals and to ensure that these guidelines are followed. It is strange to note that the CPCSEA constituted by the Ministry of Environment and Forests, Government of India, to controlling and supervising experiments on animals under the act of 'Prevention of Cruelty to Animals' has published in April 1998 new rules entitled: 'Experiments of Animals by Establishments and Breeding (Control & Supervision) Rules: 1998', which go much beyond the prevention of cruelty to animals or considerations of environment, and plan to control all research using animals. The rules framed exhibit the committee's ignorance of what animal research involves and does, and appear to be framed more to stop or stall biomedical and drug research than to promote and advance national capability and interest in these areas which are vital for national health programmes. And this coming at a stage when the scientific laboratories in India are struggling hard to compete with other countries is most disturbing. According to the suggested rules, all laboratories using animals will have to get registered with this committee. Some of the suggested rules are: 4(a) No establishment shall perform any experiment without the written permission of the committee; 5(d) Institutions shall forward the application for permission to perform an experiment in the form annexed to their respective funding agency for necessary permission. The said funding agency shall submit a monthly statement of such permission granted for approval of the Committee. 6(a) Before acquiring

any animal the establishment shall give detailed information on the kind of animal to be used, the nature of the experiment to be performed, the reasons for doing so; on completion of the experiment, the establishment is supposed to submit a report of this experiment to the committee. Most of the rules simply insist that prior permission for anything to do with animals must be taken from the CPCSEA. The committee is not equipped to do so, to say the least. Also, CPCSEA does not seem to have considered the likely effect of these processes on confidentiality and intellectual property rights which are central to the drug discovery research. Compare it with the recommendation of Paton in his book mentioned earlier on p. 158 while discussing the 'future' of animal experimentation: 'Responsibility for the conduct of an experiment must remain with the experimenter, so that it is the person most closely in contact with the animal that is responsible for its well-being'. The committee has also proposed to totally ban the import of animals for experimentation, which will be a very retrograde step for our biomedical research. In addition to naturally existing laboratory bred animals, a whole range of genetically engineered animals which simulate humans in disease conditions are now available. These animals can save much time in drug development. *CPCSEA should basically be concerned with laying down the guidelines for animal housing and husbandry and for ensuring experimental conditions to minimize pain and suffering, and monitor that Animal Ethics Committees meet and function effectively. All other functions do not belong to this committee and should be performed by the Institutional Animal Ethics Committees.*

It is difficult to imagine how such a committee can function under the Ministry of Environment & Forests (ME and F), when both the environment and wildlife are only small issues and not at all controversial. It is understandable that ME and F should be concerned with the preservation of wildlife, and can legislate that only laboratory bred animals will be used for research, but the rest does not belong to this Ministry. The correct place for a committee dealing with animal experimentation for biomedical research would be in the Ministry of Science and Technology or Ministry of Health and Family Welfare. It is amazing how a whole lot of members of this committee, including a number of very senior ex-officio member scientists purportedly have agreed to what might lead to dismantling of major components of our drug and biomedical research. The proposed rules and functions of this committee would have very far-reaching effects on the future of our biomedical research and cannot be taken lightly. It is strongly urged that a National Committee of Scientists involved in drug and biomedical research should be set up to examine the proposed objectives and rules of this

committee. The CPCSEA rules should also be circulated to all the major laboratories using animals, whether in the academic/government or private sector and their opinion should be taken into consideration by this committee while finalizing its recommendation. And, till such time as the recommendations of this committee are finalized, the CPCSEA should perform only a supervisory function to ensure that each laboratory has a functioning Animals/Human Experiments Ethics Committee in position and functioning effectively.

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