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Animal Testing

1. Humans have benefited immensely from scientific research involving animals, with virtually every medical achievement in the past century reliant on the use of animals in some way. Developments in the treatment of diabetes, leukaemia and heart surgery transplants, amongst others, have been made possible through the use of animals in scientific research. The majority of the scientific community consider that the benefits that have been provided by the use of animals in research justify this use. The public also increasingly accepts the use of animals in research. Polls have shown that 90% of the public accepted the need for the use of animals, providing that certain research conditions were met, which are: there is no unnecessary suffering; the research is for serious medical or life-saving purposes; and there is no reliable and informative alternative to their use.

2. It is important to recognise that although a significant proportion of work using animals is basic research whose benefits to health are not immediately evident, this work is highly valuable as it provides the groundwork for future medical advances. The appropriate moral stance for all use of animals in research is to minimise animal suffering and maximise the benefits to medicine and health, agriculture and fundamental understanding.

3. Animal research only takes place after careful evaluation and within a framework of robust controls. UK legislation requires that researchers refine their procedures to keep suffering to the minimum, ensure the number of animals is reduced to the minimum required for meaningful results, and seek to replace the use of animals with non-animal alternatives where appropriate. However, the use of non-animal alternatives may not be appropriate for some types of research, and these experiments may be permitted provided they are designed to keep pain and suffering to a minimum.

4. The majority of research using animals (84%) involves the use of rodents as experimental subjects, with the use of non-human primates for research purposes, which are subject to particularly rigorous control due to the special protection granted to them under the 1986 Act, accounting for less than one percent of total animal experiments. Basic research and drug development accounted for 82% of all procedures, with safety testing accounting for the remaining 18%.

5. Opponents of the use of animals in research frequently use the availability of alternative, non-animal, research methods as evidence that research using animals is unnecessary. It is true that non-animal methods, such as tissue culture, computer modelling, research using human test subjects and population studies, are frequently used and have utility for scientific and medical research. However, these methods are generally used in addition to animal studies, and do not replace them.

6. It is rarely possible to replace animals with a non-animal alternative. This is because a research study often requires more than knowing how individual molecules, cells or tissues behave. Instead, scientific research, and medical research in particular, often depends on understanding not only the processes of the living body but how they interact.

7. It is unethical and illegal to expose human patients to new medicines without being confident that they are likely to benefit and not be seriously harmed, so the only alternative is to use the most suitable animal to study a particular disease or biological function. Additionally, in drug development, animal tests are used to establish both the benefits and the potential toxicity of substances and ensure that human volunteer studies are carried out with a full scientific understanding of the substance involved, to maximise the benefits and avoid possible serious side effects.

8. Within this article, the following issues are considered: examples of medical advances that have been achieved through the use of animals; the theoretical framework behind the use of animals; the legislation that regulates the use of animals; and discussion of philosophies that underpin the debate about the use of animals in research. This article is not intended to be a manual of how to conduct research. Nor is it a substitute for good advice on experimental design, statistical analysis or any of the other aspects of conducting a successful research project.

9. The understanding of the human body has come from more than 200 years of research on the function of normal cells, tissues and organs, and on disease processes. Much of this understanding has been facilitated by research that was performed on animals. This fundamental knowledge underpins the teaching of medicine and veterinary medicine and has been instrumental in the development of medical advances for both people and animals.

10. It is no exaggeration to say that almost every form of conventional medical treatment, such as drugs, vaccines, radiation or surgery, rests in part on the study of animals. However, this fundamental link is frequently not appreciated and the following examples illustrate some of the important medical advances that have resulted directly from animal experiments.

11. Model species are used to test possibilities that would be difficult or impossible to test using the target species. In general, one species may be used as a model for another when, despite other differences between them, the two species strongly resemble each other in particular ways. Molecular mechanisms and those involved in cell differentiation and propagation are frequently identical across a wide range of species. Therefore, for some of the most fundamental principles of biology, using animals as models can provide valuable insight into human cell processes.

12. Animal models also enable a much greater control of experimental conditions than could reasonably be achieved in humans. Human patients can be highly heterogenous in disease symptoms and their behavioural variability, such as differences in patients' compliance with instructions, is far more significant in human trials. In certain uses of animals as models, such as mouse 'knock-out' models, in which individual genes are switched off to study the effect, researchers can even control the genetic make-up of the experimental subjects to ensure homogeneity.

13. Opponents to the use of animals in research claim that using animals as models for humans is invalidated by the differences between humans and animals. Evidence presented to support this argument includes the case of the unpredicted limb defects that occurred in the children of women who took the drug thalidomide during pregnancy. It is true that the damaging effects of thalidomide on developing embryos were not predicted in the initial animal experiments but this is because the effects of thalidomide during pregnancy were not looked for. Had the researchers at the time attempted to anticipate these effects, then research conducted using pregnant animal research subjects would have been able to detect these effects, as seen in later studies conducted after the effects of thalidomide in humans were discovered and the drug was withdrawn.

14. It is important to emphasise that animals are normally highly accurate models for humans, and in this instance it was experimental design that failed to look for the teratogenic effects, not that the experiments failed to detect the effects. The similarity between some animals and humans is best demonstrated by the fact that many drugs can be used to treat both human and animal patients, such as antibiotics and tranquillisers.

15. The last two decades have witnessed a revolution in biology with the sequencing of the human, mouse and fly genomes. A wealth of information has been produced about the role of genes and gene products in some diseases, as well as providing insight on why some people respond to some medicines better than others. The knowledge now available from the sequencing of the human, mouse and fly genomes has enabled genetic modification of animals to produce highly specific models of diseases, helping to identify disease pathways and aid the development of new therapies.

16. Following are three case studies that provide examples of medical advances that have been developed through the use of animals in research.

Case study on polio vaccine

17. An example of the use of animals as models of human cell processes is the development of polio vaccine. Polio is an infectious disease that can strike at any age but mainly affects children under three years old, and is prevalent in developing countries. The polio virus enters through the mouth and once it enters the blood stream can invade the central nervous system, destroying nerve cells in the limbs, trunk and the brainstem, resulting in paralysis and sometimes death. Soon after the introduction of effective vaccines in the late 1950s and early 1960s, polio was brought under control, and practically eliminated as a public health problem in industrialized countries. Research into polio vaccine requires the use of living nerve tissue to ensure that the virus used for vaccine production causes the paralysis typical of polio, and no human or tissue culture alternative is available.

18. Additionally the polio vaccine uses a live attenuated virus, which is notorious for sometimes reverting to virulence, so animals are still the only practical way of predicting the potential virulence of each batch of polio vaccine. As a result of this potential risk to humans, each batch of vaccine is tested in animals. This previously involved an intra-cerebral injection of the vaccine into monkeys, which is highly predictive of virulence.

19. More recently mice have been genetically engineered to have the receptors for the virus, providing animal models of the disease. Despite the many differences between mice and humans, the use of genetically modified (GM) mice to establish the virulence of the vaccine provides an accurate model of humans in this respect. This is a good illustration of how mice, and in particular GM mice, can be used as models for human pathogens. Besides being genetically similar to humans, mice are small and inexpensive to maintain. Their short life span and rapid reproductive rate make it possible to study disease processes in many individuals, thus gaining a greater understanding of the progression of the disease within a short space of time.

Drugs for the future

20. A great number of diseases remain for which drug therapies are far from optimal or even nonexistent. Many drug targets are proteins and the completion of the human genome project, the blueprint for around 30 000 human genes and 250 000 proteins, has enabled the genes encoding for these proteins to become known and provides potential for a greater understanding of disease at the molecular level than at any other point in history.

21. Techniques such as genomics and proteomics can help provide a wealth of information about these proteins such as their functional role, and can help refine experimental procedures, but they cannot substitute for the complexity provided by whole subject studies for increasing understanding and developing treatments of disease. The long and complex route from discovery to drug will inevitably require the use of animals at some stage of the scientific investigation and it is important to realise the limitations of non-animal techniques.

22. Many challenges to the healthcare systems of both developed and developing countries exist that would benefit from the use of animals in research. Examples include: drug-resistant infections, which are a major problem in both developed and developing countries, frequently involve examination of disease progression and research could benefit from the use of animal models such as genetically modified mice; mental illnesses, especially depression, schizophrenia and anxiety, which require a holistic approach, involving molecular, cellular and whole animal studies; better treatments for diseases of bone, joints and the immune system, all of which involve complex interactions throughout the body; treatment of diseases of genetic origin, such as Duchenne muscular dystrophy, which could utilise genetically modified animals to mimic human patients; blindness and deafness, which involve analysis of these functions in living research subjects; HIV infection and AIDS, with animal models of the disease required to develop and test possible vaccines or antiviral agents before trials can be safely conducted in humans; senile dementia and other complications of old age, where the cause and progression of these conditions is poorly understood at present, and require detailed examination of the brain and nervous system in animal models.

Case study on cystic fibrosis

23. Cystic fibrosis is an example of a disease where future therapies are being developed through the use of genomics. Much research is focused on correcting the genetic deficiencies that lead to the disease either by gene therapy, which would turn the faulty protein into a functional protein, or by finding alternate pathways to bypass the need for the protein. The cystic fibrosis gene, cystic fibrosis transmembrane-conductance regulator or CFTR, codes for a large protein, CFTR protein, that functions as an epithelial chloride channel and has a regulatory influence on many other cellular proteins. Without a functional form of this protein a person suffers from a lethal genetic disease, cystic fibrosis.

24. In other conditions, such as the secretory diarrhoeas associated with cholera, CFTR is a key player in the devastating loss of bodily fluid. The need to discover high affinity ligands that interact with both CFTR protein and some of the common faulty forms of this protein is key to the development of cystic fibrosis therapies. A recent search for CFTR ligands was carried out using cells in culture expressing CFTR protein and a halide indicator, as normal CFTR functions as a chloride channel. In a recent search for agents that activated or inhibited the channel, CFTR protein was expressed in cultured cells that also contained a reporter molecule that altered its fluorescence when either chloride (or other halide) entered or left the cells.

25. By screening 50,000 diverse compounds, six compounds were discovered that blocked the activity of CFTR protein and further chemical modification led to several channel blockers with sub-micromolar activity. Having identified these six lead compounds using non-animal methods, further research was then carried out by testing in mice to establish the most active compound. What this example shows is the multiple methodologies involved in testing a potential therapy, and that the use of animals remains a key stage. The search for CFTR activators continues, which may prove useful for future therapies for cystic fibrosis, and further investigations will undoubtedly require the use of transgenic animals bearing common mutations of the CFTR gene.

Ethical approaches to the use of animals

26. All those involved in the debate about the use of animals in research lay claim to one or other moral principle. In human and veterinary medicine, causing pain or suffering in a patient is considered unethical unless it is for the direct benefit of that patient. Those who favour work on animals may do so to alleviate the suffering of humans or other animals. Scientists in favour of this principle use their research to understand fundamental aspects of biology that in turn facilitate the development of therapeutic measures for both animals and humans. Those who oppose the use of animals in research may object to the means by which scientists attempt to achieve their goals.

27. One view is that each animal has the right to life and humans should not take such a right away from it. It is not entirely clear whether the proponents of such a view would grant rights to every organism that showed signs of reacting to maltreatment. Nevertheless, they would argue that rights to good treatment, once granted, must be respected.

28. Others would argue that while granting rights to animals is inappropriate because human rights are firmly embedded in a social context, humans have responsibilities for animals in their care and should ensure that their welfare is good. Both the rights and the responsibilities arguments are sometimes taken as absolutes, over-riding all other moral claims. However, this could also be the case for the moral argument for supporting animal experiments because of their potential medical benefit.

29. The alternative to such absolutism is to respect the range of views by attempting to both minimise the suffering inflicted on animals used in research while maximising the scientific and medical gain. This is the position enshrined in UK law governing the use of animals in research. Balancing between these positions required by law is not an exact process since the assessment of scientific and medical benefit and that of animal suffering are both difficult to quantify and are not expressed in the same terms.

30. The assessments are incommensurate and, therefore, referring to the judgement as cost-benefit analysis is misleading. So the degree of suffering might be expressed as low, medium or high and the likely scientific and medical benefit might be similarly classified. Research that involves low suffering to the animals and was likely to be highly beneficial would generally be regarded as acceptable. Research that involves medium suffering but only a medium chance of generating a beneficial outcome would probably be deemed unacceptable – but clearly this judgement will depend on a consensus view derived from a judgement by those bodies responsible for granting approval to research projects.

31. Criticism of the use of animals in research sometimes arises when there appears to be no immediate tangible health benefit of the research. An inability to quantify the benefits of a research project can be seen to imply that it is frivolous or wasteful and therefore unethical. This is an invalid assumption, however, as research studies that do not have direct benefit to humans or other animals can instead provide a vital contribution to fundamental scientific understanding that may provide benefit in the future.

32. Individual experiments are similar to the individual bricks in a building, with knowledge being built up over a long period of time and with the benefits perhaps only being realised when the building or the research is completed. On the other hand, the benefits of applied research may be easier to quantify. For example, in drug development, many thousands of compounds may need to be tested in order to develop a new drug. This means that in some cases the research may not be successful, and may seem futile, whereas in fact such work is essential in refining knowledge.

33. It is therefore important when considering the ethical justification of the use of animals in research to realise that the development of a successful drug such as insulin or the antibiotics may result in saving many millions of human and animal lives.

Ethical issues surrounding the use of genetically modified animals

34. The potential benefits of using genetically modified animals for research are great, and there is a strong scientific case for using such animals in order to understand human and animal disease. However, the use of genetically modified animals in scientific research may raise additional ethical issues, such as concerns about possible additional welfare costs that may arise from the use of this technology, and ethical questions about using animals in this way.

35. A largely hypothetical concern with respect to genetic modification is that introducing a gene into an organism from a very different type of organism may lead to unforeseen interactions in development, leading to the emergence of animals that have serious welfare problems. However the targeted genetic modification approach used to create genetically modified animals for research purposes is likely to be more predictable than other methods of inducing genomic changes, such as radiation exposure or chemical mutagens.

36. Intensive behavioural studies have been conducted on GM animals to monitor any adverse welfare implications and these have found very little difference between animals that have been genetically modified and those that have not. Moreover, we should also bear in mind that animal breeders have been selecting for genetically determined traits for many centuries, such as the range of dog breeds, which are widely accepted but raise similar ethical and welfare issues to genetically modified animals.

Refinement, reduction and replacement in animal research

37. The guiding principles of animal welfare are the so-called 'three Rs', refinement, reduction and replacement, first clearly defined in 1959. This means that every effort must be made:

- to **refine** the procedures so that the degree of suffering is kept to a minimum.

- to **reduce** the number of animals used in research to the minimum required for meaningful results.

- to **replace** the use of live animals by non-animal alternatives.

38. Current UK legislation requires all researchers who propose to undertake laboratory or fieldwork involving animals to give full consideration to the three Rs and to seek independent advice and approval from a local ethics committee. The approvals process for personal and project licences emphasises that researchers should seek, where possible, to avoid the use of animals and must advance sound and detailed scientific arguments for their use, explaining why no realistic alternative exists. Scientists must also address what is the acceptable trade off between the welfare benefits of the three Rs approach and the costs of not obtaining a more definite result through using intact animals.

39. The principle of the three Rs is not to remove the use of animals in research, but instead to provide a mechanism to ensure the best possible use of animals in research. Using animals in scientific research provides a bedrock for increasing understanding, and is consistent with the three Rs as developing our knowledge helps to enhance existing techniques using animals, as well as generating knowledge that underpins research studies using humans.

Refinement

40. Refinement is defined as 'reducing to a minimum the incidence or severity of suffering experienced by those animals which have to be used'. Refinement of experimental methods, for instance through adequate post-operative care, good housing, and improved anaesthesia and analgesia has been standard practice in biomedical research for many years. Refinement of surgical procedures is frequently by technical advances but also by experimenters asking themselves some generic questions that can be asked prior to undertaking any invasive procedures: can I minimise the area of tissue at risk of damage or infection? Can any aspect of the surgical process and subsequent recovery be made less troubling to the animal?

Case study on refinement

41. A common question directed to researchers using animals is how can one be sure that the species being used is appropriate to the study of human health or illness. Selecting the appropriate model is an important step scientifically of course, but also in terms of animal welfare. The more one can predict the course of the disease induced in the animal, the better one can anticipate the animal's behaviours and needs such as changes in housing, diet or diurnal routine. Use of a certain species for research purposes is dependent on the process or disease under study. If one compares the genomes of the mouse and humans, one finds that there is a mouse homologue for 99% of human genes, and of the genes implicated in human disease processes, 90% are present in the mouse. As a consequence of this degree of similarity, major advances have been made recently by the development of transgenic mouse models of human neurodegenerative disease.

42. The identification of mutations in the SOD1 gene as the cause of Amyotrophic Lateral Sclerosis (ALS), a lethal neurodegenerative disease, has opened new possibilities. Mice with mutations in the SOD1 gene develop severe motor neuron degeneration similar to that seen in humans and targeting specific mutations in SOD1 has given researchers the ability to study specific symptoms such as limb weakness, axonal swelling and the timing of onset of symptoms. Genetic manipulation of the mouse is constantly being refined and a future possibility is to be able to switch genes on or off at different stages of the animal's life – a critical step in the study of late onset neurodegenerative diseases. As a result of the experimental refinements made possible by what is known as the 'genotype driven approach', gene therapies for ALS and other motor neuron degeneration diseases (all of which are incurable) have begun to be developed.

Reduction

43. A requirement of the Animals (Scientific Procedures) Act 1986 is that the smallest number of animals should be used, consistent with achieving the objectives of the experiment. The number needed will depend on the variability of the animals, the minimum size of any statistically significant difference between treatment groups and the chances of obtaining misleadingly negative conclusions.

44. Using animals of similar age, weight, genetic composition and so forth can reduce variability of the animals. Best use of the animals can be obtained by appropriate experimental design and by correct statistical analysis of the data. Failure to use good design will result in more animals being used than is necessary. Poor statistical analysis will result in unnecessary waste because the conclusions are unreliable or the data are not used as productively as might otherwise have been the case. This means that researchers must be properly trained in statistics and should receive advice from a statistician, who is experienced in dealing with those who work with animals.

45. Sound experimental design and forethought about statistical analysis are the first steps to reducing the number of animals used in any procedure. Achieving a reduction in the number of animals used is more difficult, however, when a small number of experimental subjects would have been used anyway, for example in research using non-human primates. The reduction process, like refinement, is led by generic questions: How can more data per animal be obtained? Can the experiment be repeated with the same research subjects experiencing a variety of experimental variables?

Case study on reduction

46. The introduction of new techniques can help reduce the numbers of animals used. In experiments to investigate brain function, for example, a region of brain tissue may be permanently removed by surgical lesion in an attempt to understand its function. It may be possible, however, to use reversible chemical lesions, or temporarily interfere with brain function through cooling by injecting chilled saline into the study area (Lomber 1999) to decrease the numbers of animals used. Brain images of animals also provide information about the area under study, and may help to reduce the need for more invasive investigative methods. It may also be possible to use advanced surgical techniques that obviate the need to kill the animal, as seen in the use of partial lesions to produce visual deficits in only one quadrant of the visual field. Scientists were thus able to use the animals as their own controls by testing their perceptual functions in both the lesioned and non-lesioned part of the visual field representation. These experiments were also able to maximise comparability with human data by training the animals in tests identical to those used with human subjects. Thus the amount, quality and comparability with human data were all enhanced at the same time as the total number of animals used was reduced.

47. Genetically modified animals may also offer new opportunities for refinement and reduction. A question often asked of animal research is how one knows whether the species being used is relevant. GM animals provide a means of ensuring that the characteristics of the system under investigation are as closely matched as possible to the human system that is the target of the research. The research and potential medical benefits of GM animals are already being pursued in research on heart disease, cancer and muscular dystrophy. From a refinement point of view, having available the most appropriate animal model increases the power of an experiment and also allows the most sensitive techniques and measures to be used. These experimental refinements can provide answers to the three questions posed above: the best choice of animal, which may or may not be GM, is a first step to minimising unwanted aspects of experimental procedures.

Replacement

48. The use of whole animals is a key element of much scientific and medical research as it enables normal physiological processes to be studied within the environment of the living body, and helps identify interactions that influence disease processes.

49. When a specific mechanism can be identified, the use of cell cultures may be possible. Similarly, molecular sensors may be used to test the biological activity of particular substances. When enough is known about a complex system found in intact animals, computer simulations could prove helpful in exploring the dynamics of that system; in effect, experiments can be conducted on the computer model. However, such studies generally suggest and require further work on whole animals and do not completely replace experiments on animals. Alternatives to whole animals are clearly versatile, but are as yet incapable of capturing the complexity of the living mammalian body. Research that would be typically carried out on animals has been conducted on humans, but when the outcome is unknown, as is usually the case in research, or the techniques are invasive, the ethical justification for such work is highly questionable – even when the human subjects are volunteers.

Case study on replacement

50. The lesion method, studying the effects on behaviour of compromising or removing tissue, is a widely used experimental system in behavioural sciences. One can, however, temporarily interfere with brain functions by applying brief magnetic pulses to transiently interfere with normal function. This method therefore replaces the need for permanent, surgical interference of brain function in some experiments. In practice, this technique, called transcranial magnetic stimulation or TMS, has now been used to study the timing of information transfer between human cortical areas, changes in brain function due to learning and a wide range of perceptual, movement and language functions.

51. This ability to use human subjects in lesion experiments now obviates the need for some lesion experiments in non-human primates. This is limited, however, to cases where the brain region of interest is accessible to surface stimulation. Many areas of interest lie too deep to be penetrated by the magnetic fields, in which case surgical lesions in animals are still necessary. A question about TMS is how one knows the right area has been stimulated with the same degree of certainty as total removal of a brain region by surgery. However, high levels of accuracy of the TMS technique can be established by co-registration of the magnetic stimulation site with a human subject's individual MRI scan. TMS also has very high temporal resolution and cortical functions can be disrupted within time windows as small as 5 milliseconds, thus allowing a temporal dimension to lesion analysis that is difficult to achieve in monkeys with other methods.

52. The principles behind these examples of the three Rs can be extended or modified to other types of animal research. Reduction would seem to be the factor on which most progress can be made by changes in experimental design and procedures. Refinement is an ongoing process dependent on new techniques and improvements in husbandry. Researchers routinely implement refinement and reduction in their daily routines – it is both good science and cost effective to improve experimental design and increase statistical power. The third R, replacement, is more difficult to achieve because of the unique insights provided by use of whole animals and because some techniques cannot of course be used with human subjects. Notwithstanding the difficulties, every effort to pursue the three Rs should be made.

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