

# Is the Use of Animals in Biomedical Research Still Necessary in 2002? Unfortunately, “Yes”

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**Summary** — The use of laboratory animals in the year 2002 is essential both to maintain human health and to develop new treatments for the many diseases that still plague humans. The suggestion by Greek and Greek in *Sacred Cows and Golden Geese* in 2000, that animal experiments are invalid because animals are different from humans, shows clearly that they do not understand the philosophical basis for the use of models in science and every day life. Models only need to resemble the thing being modelled (the target) in a few key respects. A map of Brooklyn Botanic Garden is a useful model, but it differs from the garden in many respects. There are many examples where studies on animals and *in vitro* alternatives result in accurate predictions of human responses even though the models differ from humans in other ways. In the drug development model, validation is done in clinical trials. Models are also used in the discovery of fundamental processes shared by some, or all, living organisms. The laws of genetics were first discovered by using garden peas, but they are equally applicable to humans. It is because of the ethical, rather than scientific, objections to the use of animals that all scientists are urged to find alternatives according to the principles of *reduction*, *refinement* and *replacement*, laid down by Russell and Burch in 1959.

**Key words:** *animal experiments, animal models, reduction, refinement, replacement.*

## Introduction

Greek & Greek (1) claim that because animals and humans are different, “Animal models are inaccurate, superfluous, and create risk to humans”. They suggest that there is no objection to veterinary research, but it must be done separately for each species. Somewhat similar claims are made by a group called Europeans For Responsible Sciences, who have stated that: “. . . the very idea that one species could serve as a model for a different species ignores the basic principles of biology”. They go on to say that: “. . . the species’ response to any external stimuli (toxic products), or to internal dysregulation (pathologies), are strictly species-specific also. These facts rigorously demonstrate that no species can be seriously considered as a biological model for another species, no matter how closely related they are in evolution”.

Unfortunately, both these claims are untrue. They are based on wishful thinking, a misunderstanding of evolution and the rejection of any evidence that does not support their claims. Were they right, these arguments would also apply to most alternatives to the use of animals. After all, a mathematical formula, a computer simulation, or cells growing in tissue culture are even more unlike humans than are laboratory animals, yet such models are often successfully used in research. And the claim that no species can be used as a model for a different species is also invalid. If this were the case, a veterinarian needing to treat an exotic animal such as a tiger, would have no drugs or anaesthetics available, because none have been developed in tigers for tigers. In fact, there are a wide range

of anaesthetics, drugs and antibiotics that are available to zoo vets, provided that they are used with care. There are species differences in response, but these are relatively insignificant. The fact is that the continued use of animals is essential both to maintain human health by the production of vaccines and pharmaceuticals, and to support research into the many serious diseases that still plague society. What animal welfare organisations should be doing, and many are doing, is to develop alternatives that do not also put human lives at risk. Russell & Burch’s Three Rs provide a framework for progress (2).

The enormous contributions that animal research has made to our understanding of human biology and the development of medicine have been discussed elsewhere (e.g. 3, 4). All good scientists now accept that every animal experiment must be scientifically justified and the cost to the animal in terms of pain and/or distress assessed. If the estimated cost to the animal is large in relation to the potential benefits to humans, then the research should not be done. The use of animals to test cosmetics, for example, is already banned in the UK. The aim of this paper is to show how animals (and alternatives) can be used to model humans, even though they may differ from humans in many ways.

## Animals and *In Vitro* Methods as “Models” of Humans

There is no dispute that humans and any species of animal differ in many thousands of ways. The same

is true for *in vitro*, mathematical and computer models of humans. Therefore, the philosophical basis for the use of models needs to be considered. According to the American philosopher Marx Wartofsky (5):

*Theories, hypotheses, models and analogies I take all to be species of a genus, and my thesis is best stated directly by characterizing this genus, as representation (though 'imaging' or 'mirroring' will do quite as well).*

Thus, animals and *in vitro* alternatives are "representations" of humans. Wartofsky goes on to say that:

*There is an additional trivial truth, which may strike some people as shocking: anything can be a model of anything else! This is to say no more than that between any two things in the universe there is some property they both share, . . .*

This statement is of critical importance. The model and the thing being modelled (the target) can differ in many millions of ways, but they only need a single characteristic in common for the model to be a valid representation of the target in some specific respect. It is this specificity that is important. This point can be illustrated by an analogy. A map of Brooklyn Botanic Gardens is clearly a *representation* or *model* of the true botanic garden. However, the two differ in many millions of ways. The real garden has plants, soil, insects, water and buildings, none of which are physically present on the map. The map consists of paper and ink, which are not a major component of the real garden. In fact, the only similarity between the map and the real garden is the spatial layout of the main features of the garden and their representations on the map. Thus, there is asymmetry between the very few similarities and the many differences between the two.

Models are developed for specific purposes. The map is only useful for showing the relative location of features in the garden. It is of no value for growing plants or getting a cup of coffee, both of which can be found in the real garden. Models often use a different scale from the target. The map is much smaller than the garden, and would be useless if it were full size. A model of DNA, in contrast, might be substantially larger than the real molecule, so that people can easily see and understand its structure. Thus, a model *must be different* from the target; otherwise, it would not be a model. Simply bringing attention to these differences, as done by the Greeks with respect to laboratory animals, does not imply that the models are invalid.

Models generally need to be validated. There needs to be some assurance that they are useful for the purpose for which they are designed. If the features shown on the map do not correspond with those in the real garden, then the user would suspect that she

has the wrong map, or possibly does not know his or her present location. Similarly, both animal and non-animal models used in biomedical research need to be validated sooner or later to ensure that the predictions that they make are valid. This can be done formally (6) or informally. Clinical trials of new drugs, for example, provide one way of validating the predictions of animal models used in drug development. If the models turn out to be invalid, the drug will not be successful.

Models can also be used to derive new information that is either impractical or impossible to obtain from the target. An individual given the map can immediately locate the restaurant or the glass houses. It might take them hours to find these features by walking through the real garden. There seems to be some confusion on this issue. In discussing the nature of models LaFollette & Shanks (7) state:

*Yet neither activists nor researchers should demand or expect that animal models be representations of human biomedical conditions. For if animal models were mere representations, then they would be useless to the scientific enterprise. Since a model Boeing 747 is manufactured to embody the details of a real Boeing 747, then we cannot learn anything new about the real plane by looking at its model — it is a model only because we made it similar to the thing it models.*

It is not clear why these authors assert that animals are not "representations" of humans when this seems to be exactly the way that they are commonly used. This will be discussed later. However, their claim that a model of a 747 cannot tell us anything new about the real 747 is clearly wrong. Models are widely used in the manufacture of airplanes. A small-scale model of the proposed plane will be used in wind tunnel tests of its aerodynamic characteristics. Wind tunnels cannot accommodate a full-sized airplane. A similar model could be used after the 747 has been built to improve these characteristics. There will certainly be full-scale models of the internal cabin to test out seating and evacuation procedures. There will also be a flight simulator to train the pilots before they are let loose on a real airplane. These models all provide new information that is impossible or impractical to obtain from the real 747. In the same way, *in vitro*, invertebrate and vertebrate models of humans can certainly be used to gain new information that is impossible to gather directly from humans, as discussed below.

## Two Examples of the Use of Models in Predictive Tests

Models are often used for directly predicting human responses. The Ames test is an example of how bacteria can be used as a model of humans (and other

species) for the specific purpose of predicting whether a chemical is likely to be carcinogenic. Chemicals that damage DNA can cause mutations, and in animals, these can lead to cancer. Bacteria, like animals, have DNA, and it is reasonably easy to determine whether a given chemical causes mutations in bacteria. It would be much more difficult to detect such mutations directly in humans, because they would need to be exposed to the chemical, which would often be unacceptable. Epidemiological evidence can sometimes be used, but only when humans have already been widely exposed, and even then, the effects will only be detectable if they are large. So the special strains of highly sensitive bacteria in the Ames test are used as a model or representation of humans for this specific purpose. The Ames test has been validated by testing it with chemicals believed to be carcinogens and non-carcinogens. It is not particularly accurate, but used in conjunction with other *in vitro* and *in vivo* tests, it is considered to be useful, at least, in eliminating from further development many potential pharmaceuticals that cause mutations. Thus, although bacteria and humans differ in many thousands of ways, for this specific purpose, bacteria can be used as a model of humans.

Similarly, rabbits were used in the development and assay of insulin (8). Relatively pure insulin was isolated in about 1922 from bovine and porcine pancreatic tissue by the chemist, J.B. Collip, working with Banting and Best. Collip had difficulty in isolating insulin and tried out many different methods of fractionating the pancreas extracts. He needed a method of determining which fractions had insulin activity, and he discovered that when insulin is injected into normal rabbits, it reduced blood glucose levels in the same manner as when it is injected into humans. It simply was not acceptable to use humans for this purpose. Collip and the pharmaceutical company, Ely Lilly, used over 100,000 rabbits in establishing the commercial production of insulin. Later, the rabbit assay was used to assess the potency of different batches of insulin.

Although rabbits and humans differ in many ways, they do not differ significantly in their response to insulin, so rabbits could be used as a scientifically valid model of humans for this specific purpose. Insulin differs slightly between species, but has not evolved to the extent that the responses are species-specific. Bovine and porcine insulin work in humans. Fortunately, a physicochemical, HPLC method was subsequently developed, and animals are no longer needed for assaying insulin (9), which, in any case, is now human insulin grown in genetically engineered human cell lines, rather than being extracted from animals. This is typical in that animal assays used in the early stages of a research project can usually be eliminated by the use of non-animal alternatives, although, this may take from a few weeks to many years.

### Three Historical Examples of the Use of Animals as Models in Developing Treatments for Human Disease

There are numerous historical examples where the use of animals has been essential in developing treatments for human disease. These are discussed below, not just to show that the use of animals has been successful, but also to show how and why this is the case with brief comments on why human studies could not have achieved the same results.

#### A rabies vaccine developed in dogs and also worked in humans

In 1880, Pasteur developed an anti-rabies vaccine by using dogs. This can be regarded as a clinical veterinary research project involving the use of dogs to develop a vaccine for dogs. Greek & Greek (1) accept that clinical research within a species can be successful. First, Pasteur developed a reproducible way of inducing rabies in the dogs. This he did by using intra-cerebral injections of infected nervous tissue. Next, he developed a vaccine, which involved attenuating the virus (viruses had not been discovered at that time, so he did not know what sort of organism he was dealing with). His method seems to have been based largely on guesswork. He dried the spinal cords of infected rabbits for up to 14 days (presumably, he could equally well have used dog spinal cord, as claimed by Greek & Greek [1]). He then used a homogenate of 14-day, 13-day, etc. dried spinal cords given over a 14-day period to vaccinate the dogs, and was successful in immunising 50 dogs, so that they were resistant to the virulent virus. This research took about five years, and it showed that his vaccine worked in dogs, but he did not know whether it would work in humans. However, at that time, he was faced with a child who had been badly bitten by a rabid dog, and he was implored by the parents to use the vaccine. In doing so, Pasteur recognised that he was taking a great risk. Had the boy developed rabies, Pasteur might have been accused of causing it with his vaccine. Fortunately, the boy did not develop the disease, and among the first 350 people that he subsequently treated, only a single person developed the disease, and she had not been vaccinated until 37 days after being bitten. Even so, he was accused of causing rabies in this case (10). It has been estimated that somewhere between 40% and 80% of people bitten by rabid dogs develop rabies, so there is not the slightest doubt that Pasteur had in fact developed a highly effective human vaccine, which has since saved many thousands of human lives. It was not without problems. It did sometimes cause allergic reactions and was often extremely painful.

However, most people would consider that acceptable, given that rabies is invariably fatal once symptoms develop. His vaccine continued to be used until after World War II, but has now been replaced by a vaccine prepared in cell culture.

While Greek & Greek (1) may be surprised that a vaccine developed in dogs also worked in humans (in their book, they do not even admit that Pasteur developed a rabies vaccine), most scientists would not consider it in any way strange. Dogs and humans are both susceptible to rabies, and both have an immune system that can develop immunity to viral diseases. Clearly, it would have been impossible to develop such a vaccine directly in humans, because Pasteur had no assurance that his vaccine would work, and it might have caused rabies. Imagine, for example, that Pasteur thought that dried spinal cords from dogs or humans who had died of rabies could be used as a vaccine. Somebody is bitten by a rabid dog, and the vaccine is given to them. If they then got rabies this could be because the vaccine caused rabies, or because it was ineffective in preventing it. The two could not be distinguished. However, if they failed to get rabies, this might have been because the vaccine was effective or because the bite did not result in rabies. Again, the two could not be distinguished. Given the very real danger that the vaccine could have caused the disease, clinical trials in humans without the preliminary experiments in animals would have been out of the question. But the dog model was subsequently validated by clinical use of the dog vaccine in humans.

### **Chemotherapy of syphilis developed using rabbits**

In about 1907, Paul Ehrlich developed the hypothesis that certain arsenic compounds might be able to kill the organism (*Treponema pallidum*) that causes syphilis without killing the patient. He screened over 600 such compounds *in vitro*, but none of them showed significant promise as drugs for treating the disease. However, in 1909, a colleague in his laboratory had managed to infect rabbits with syphilis, and he was able to use this animal model to screen these chemicals again. One of these, later named Salvarsan, was effective against the organism in the rabbit model, and was subsequently found to be effective in humans. This revolutionised the treatment of syphilis, though, as a drug, it had many disadvantages and was subsequently replaced by Neosalvarsan and later by antibiotics (11). Again, it is very difficult to see how this could have been done using syphilitic humans. The sheer logistics of working out dose levels, medical histories and treatment regimens for hundreds of syphilitic patients presumably at various stages of the disease, with no prior suggestion that any of

the drugs were effective, would have made the research impossible.

There was, of course, no assurance that Ehrlich would be successful. But failure with an animal model is less serious than failure of a clinical trial in terms of human welfare and research resources. Had the animal model failed in this case, it could have been due to two possible causes. First, the model could have been inappropriate, so that even if there were useful agents it would have missed them. Second, the model may have failed because useful agents are so rare. In this case, only a single useful compound was found among more than 600. Failure in this case would not be attributable to an inappropriate model, but rather to the sheer difficulty of finding useful agents. In these circumstances clinical trials would also have failed.

### **The development of penicillin**

The third example is the isolation of penicillin, which was originally discovered by Alexander Fleming in 1929, though he was unable to purify it. This was eventually done by Chain and Florey in 1939. As penicillin kills bacteria *in vitro*, this purification did not require the use of animals. However, once purified samples were available, it was necessary to determine whether it was toxic, and whether it was active *in vivo*. This was done by using mice in a classical experiment described by Medawar, as follows (12):

*. . . eight white mice received approximately eight times the minimal lethal number of streptococci. Four of these were set aside as controls, but four others received injections of penicillin — either a single injection of 10 milligrams or repeated injections of 5 milligrams. The mice were watched all night (but of course). All four mice unprotected by penicillin had died by 3.30 am . . . Next morning, Sunday 26th. May, Florey came into the department to discover that the results of his experiment were clear-cut indeed. All four control mice were dead. Three of the treated mice were perfectly well; the fourth was not so well — though it survived for another two days . . . They all recognised that this was a momentous occasion . . . Animal experiments on a much larger scale soon made it clear that penicillin was indeed of great potential importance.*

In retrospect, it might have been possible to test penicillin directly in humans, but it would have been extremely risky. A substance known to be toxic to bacteria might well be toxic to humans, and it might have been difficult to determine whether it was successful. The real triumph of this research was the use of an *in vitro* model in the purification of the antibiotic. A Petri dish of bacteria is not very obviously a model of a human, but in this case, in

spite of the differences, it was useful in determining which fractions of the “mould juice” contained the active principle.

### Other Examples of the Need for *In Vitro* Models and Animals in Predictive Tests

There are many other examples of the use of *in vitro* and *in vivo* models for predicting human responses, as well as many examples of animal tests that have been replaced by *in vitro* tests. In most cases, the *in vitro* model is less like a human than the animal model that it has replaced, but these differences do not invalidate the *in vitro* model, because the differences are of no scientific significance. Currently, for example, every batch of diphtheria, tetanus, haemophilus B, pertussis, polio and rabies vaccines still needs to be tested in laboratory animals for safety and potency. The polio vaccine, in particular, can revert to a virulent form. If batches were not tested, then an occasional batch could cause polio rather than preventing it. Were this to happen, parents would refuse to have their children vaccinated, and polio would return as a serious disease. Pregnancy testing is a good example of how an animal test using mice or frogs has been replaced by an *in vitro* model (the home pregnancy testing kit) that is so abstract that it is hardly recognisable as a model of a part of the human female reproductive system, but that is essentially what it is.

Another recent example is the use of pigs as a model of humans in the development of a microwave surgical method for treating patients with terminal liver cancer (13). Colon cancer can usually be treated successfully unless it has already metastasised to the liver, which may have happened by the time that the colon cancer is diagnosed. Surgical treatment of liver tumours, however, is extremely difficult. Recently, a new microwave probe has been developed, which is about the size of a pen. The aim is to insert this into the middle of a tumour and give a dose of microwaves sufficient to kill it. It was necessary to determine what dose of microwaves would be needed to treat a tumour of a given diameter, and whether such a treatment would be likely to kill the patient. The model had to be about the same size as a human, because scaling was not possible, so pigs were chosen. These were anaesthetised and the probe was inserted into several lobes of the liver and various doses of microwaves were given. The pigs were then allowed to recover, but were killed at various times post-treatment to measure and determine what happened to the lesion. From the resulting information, it was possible to work out a dose–response relationship to determine the dose needed to treat a tumour of a given diameter in humans. The lesions began to shrink after about two months, and in no case did this treatment appear to harm the animals, though one pig died from unrelated causes.

This model is not exact, because, in humans, the probe would be treating a tumour in the liver, whereas there were no tumours in the pigs. Also, pig and human liver may have slightly different properties with respect to microwaves. Thus the first patients were taking some risks in accepting the treatment. However, as they had what was judged to be terminal cancer, they presumably considered that they had little to lose and possibly much to gain. To date, nine “terminal” patients have been treated successfully and appear to have been cured.

### But Christopher Columbus Didn't Have a Map: Models in Exploratory Research

The examples quoted above show how models, whether *in vitro* or *in vivo*, can sometimes be used to predict responses in humans, based on some knowledge of human biology. In each case, the models are used for a specific purpose, and they have been formally or informally validated as being predictive for humans, possibly in a clinical trial. The accuracy of prediction varies, but even models that are relatively inaccurate are sometimes useful, particularly when used in conjunction with other models. This is not to claim that all models are equally good, or that all research using models is successful. Lack of success may be because the model is inadequate, or because there is no easy solution to the problem.

However, models are also used for an entirely different purpose, namely, to discover new information that is unobtainable from direct experimentation on humans. A large part of research on other life forms is exploratory in nature. Basically, an organism is studied and a phenomenon is found that the investigator feels may have some general and/or biomedical significance. This is then checked to see if it is observed in other species, including humans. If so, new information is gained. If not, then it is assumed that the phenomenon is species-specific.

There are literally thousands of examples where such research has been highly successful. Just a handful of examples from the science of genetics are given here. The laws of genetics were discovered by Gregor Mendel in 1865, by using garden peas. These laws have proved to be just as relevant to humans. Muller discovered that radiation causes mutations by using the fruit fly *Drosophila melanogaster*, and we now know that this is equally true in humans. The one-gene-one-enzyme hypothesis was formulated by Beadle and Tatum in 1941, by using *Neurospora*, a fungus. Fundamental processes, such as what genes actually do tend to be universally true in all life forms, and, of course, DNA, in the form of Watson and Crick's double helix, is the blueprint for all life forms above viruses. The three base-pair genetic code is also virtually universal. A large fraction of fundamental knowledge about genetics has come from studies with *Drosophila*. More recently there has

been extensive research using the nematode, *Caenorhabditis elegans*, which is already producing information on fundamental biological processes, such as apoptosis (programmed cell death), which is of potential importance in cancer research.

### A Specific Example: The Use of Animals in Obesity Research

Obesity has now become a serious biomedical problem in developed countries such as the USA and the UK. This is probably due to a combination of the ready availability of high-energy food and a sedentary lifestyle. It is associated with type II diabetes, increased incidence of cancer and heart disease, reduced life-span and a generally poorer quality of life. Billions of dollars are spent annually on medical treatments, diets and health foods, but the problem persists. There are no satisfactory methods of treating this condition at present. It appears to be almost impossible for most individuals to control their body weight over a long period of time. Even if they succeed in losing weight by dieting, it is very rare for them to maintain their reduced weight for more than a few years. We badly need methods of tackling this problem.

The obese mouse is a spontaneous recessive mutant that arose in the late 1940s. The animals become obese from about four weeks of age due largely to excessive food intake. However, even if they are rationed to the same quantity of food as their normal littermates, they still become obese. This is because they maintain a lower body temperature than normal, and the energy saved in this way is stored as fat.

Until recently, this mutant could have been classified as an "orphan" model in that human obesity is controlled by many genes and there was no known genetic equivalent of the obese mouse in humans. However, when the mouse mutant was cloned it was found to code for a hormone secreted by adipose tissue that was named "leptin". The mutant mice were leptin deficient, and when leptin was injected into these obese mice, their appetite decreased dramatically. The human leptin gene was soon identified, and a family in which two children were obese due to leptin deficiency was found. Thus, the mouse model did, indeed, predict a human condition; however, it turned out to be quite rare. Most human obesity is not due to leptin deficiency. However, the discovery of leptin was a major breakthrough because it was the first natural substance to be found that controlled appetite, and it provided a strategy for investigating obesity through the use of obese mouse mutants, several more of which were available. It appears as though the control of appetite is complex (14), but is well conserved in evolution. Other hormones have been discovered including a peptide PYY3-36, which is

produced in the gut. When this peptide, derived from pigs, was injected into mice, rats and humans, it had the effect of drastically reducing appetite (15). Thus, as a result of exploratory animal research some progress is at last being made towards developing medical treatments for human obesity, although it may take several years before good drugs are available.

### The Numbers Game

Although the numbers of animals used in biomedical research worldwide number several millions, these numbers do not appear excessive when considered in relation to the human population. An average person in the UK living for 75 years will have had less than four animals used on their behalf by medical research. This number is made up of 2.4 mice, 0.8 rats, 0.4 fish and 0.4 of all the other animals combined. This includes all uses of animals, including those used for veterinary studies, vaccine testing, and to protect the environment. The UK has a large pharmaceutical industry and is a net exporter of pharmaceutical products, so these low numbers are not a result of importing pharmaceuticals. These numbers represent < 2% of animals used for food.

### Discussion

The Siren song of Ray and Jean Greek is highly seductive to those who campaign against the use of animals in research. If all animal models are scientifically invalid, their use could be banned immediately without any consequences for human health. Unfortunately, the Greeks are wrong. Currently, animal research is absolutely essential both for maintaining human health (e.g. in the testing of vaccines) and for the development of new treatments for the many diseases that still plague the human race. This is not to say that all animal research is essential, or that all of it is scientifically valid. There are good and not-so-good scientists, and ethical review processes do not always work perfectly. And the best scientists agonise over the scientific validity of both their *in vitro* and *in vivo* models. Research to develop new treatments is extremely difficult, but some animal research could undoubtedly be replaced by non-animal alternatives that may already exist or could be developed with a little more effort. The job of animal welfare organisations must be to develop an environment in which the potential value of every animal project is critically assessed, and no project is allowed to go ahead unless it can be scientifically justified and does not cause excessive suffering. In short, we should continue to promote the Three Rs as vigorously as possible.

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