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Replace, reduce, refine: Why do we still need to experiment on animals?

Introduction

The use of animals in scientific research has been a matter of public concern for decades. It is an emotive, complicated and often polarising topic. Animals and their products are used in peoples' diets and clothing, owned as pets, and used for activities ranging from sport and leisure, to education and work. Their use in these settings is also highly contentious, whereby some believe that animals should be granted the same rights as humans, whilst others feel that there need not be any ethical considerations attached to animals whatsoever, and that their status in society is irrelevant.

Research on animals can be defined as animal experimentation for basic and applied scientific research, as well as testing for product toxicity[1]. The issues regarding the necessity, justification and acceptability of animal experimentation are widely debated and are, unsurprisingly, extremely complex. It is clear that the contribution of animal research to our understanding of biological processes and the development of treatments for human and veterinary diseases is important. However, not all testing is undertaken to advance medical treatments or is transferrable to humans, and several new technologies offer alternatives that could theoretically circumvent the need for animal testing altogether.

Several polls[2] have indicated that animal research is more acceptable to the public if: 1. there is a defined purpose linked to human health that could not be obtained by other methods ***and*** 2. animal suffering is minimised[3]. These ethical considerations are supported by the law, which forbids studies that result in "severe, unalleviated and enduring pain or distress"[4]. Despite the fact that standards of laboratory animal welfare in the UK are some of the best in the world[5,6], there remains considerable impetus for methodologies that replace, reduce and refine animal research – *the Three Rs*[7].

This essay will explore to what extent research could realistically be conducted without causing pain, suffering, distress, or lasting harm to animals.

The Three Rs

The "3Rs" principle of "replacement, reduction and refinement" was conceived as a framework for more humane animal experimentation and testing[8].

Replacement

Replacement refers to methodologies which directly replace or avoid the use of animals in scientific research. Replacement can be full or partial[7,9]. Full replacement completely avoids animal use by using *in vitro* techniques including tissues/organoids and established cell lines, or computer modelling. Conversely, partial replacement can comprise *in vitro* experiments conducted after the animals are culled, or a combination of both *in vitro* and *in vivo* techniques using live animals.

Extensive replacement studies have been applied to the Draize eye-irritancy test[10], which involves placing a substance directly into the eye of a conscious rabbit. There was much pressure for this test to be abolished not only because of the suffering and distress it causes but also because of the inherent anatomical and physiological differences between the human and rabbit eye: the presence of a third eyelid, a thinner cornea, and a smaller volume of tear fluid. Not only are rabbits' eyes more sensitive, but this test is also based on observation only and does not yield quantitative toxicity data. Nowadays, any substance used in this test needs to have undergone previous *in vitro* tests to assess its irritability (partial replacement). Furthermore, many of these tests are now carried out only using tissue and organ donors, and scientists have also devised protein solutions that are responsive to irritant and corrosive chemicals (full replacement). A further example of full replacement is that of the production of monoclonal antibodies; initially produced using mice, these can now be produced *in vitro*[11].

Reduction

The principle of reduction is based on the premise that if animal experimentation is necessary, then the number of animals used and the number of experiments conducted must be reduced to a minimum without affecting the outcome of the research[7,9]. This can be achieved by improving the efficacy of the experimental procedures; the use of technologies that enhance the observational studies; and by sharing data and resources between research groups and other institutions[12]. To achieve this, scientists are expected to perform thorough background research to ensure their experiments are not a direct repeat of previously published work, and that the question being asked is refined to minimise the numbers used.

Regrettably, a survey of 78 experiments published in leading scientific journals showed that roughly 33% of animal experiments involved a larger number of animals than necessary[7]. To avoid this, researchers need to be sufficiently trained, and the correct equipment and methodologies chosen. This will not just minimise the level of animal suffering and loss, but careful planning of this kind will also increase the impact of the experiments. Additionally, the use of novel techniques such as longitudinal measurement imaging and venous micro-sampling enables scientists to use less animal material and to avoid repeats[9].

Refinement

Refinement refers to "methods that minimise animal suffering and improve animal welfare"[7,9]. This includes factors such as housing, food and the use of pain relief methods and training to minimise fear. In addition, animals should be given the ability to interact socially in a comfortable environment. This is not only of moral imperative: animals living in unsuitable or highly artificial environments express heightened stress levels and altered physiological and immunological functions, thus impairing the reproducibility of the results and demanding the use of a larger number of animals to achieve the same level of reliability[7]. This illustrates that a degree of compromise is necessary, whereby refinement must trump reduction, as a poorly conducted and unreproducible experiment will require repetitions which may ultimately lead to the use of more animals overall. Refinement also involves careful selection of the animal species, sex, and age for each study, whilst taking into account the adverse effects it suffers. Finally, after-care needs to be duly considered, following recommendations from vets and other health professionals[8]. To implement these strategies, scientists need to understand the sources of direct and indirect pain, suffering and distress, and all researchers need to undertake appropriate training.

Whilst all three principles should be followed, the COVID-19 pandemic has exemplified how extreme prioritisation of the 3Rs can impede scientific progress. The Netherlands' grand ambition to "*lead the way in the international transition with animal-free innovations"* has primarily resulted in an overwhelming administrative, bureaucratic, and financial burden that has limited the ability to respond rapidly to changing and emerging biological threats (such as SARS-CoV-2), whilst also reducing the quality and translatability of the published research[13]. This is not the goal of biomedical research, particularly when the lives of millions of people are at stake. Thus, more objective, fact-based, and collaborative discussion is needed.

Scientific rationale for animal experimentation in human testing and research

Animals can be very useful models thanks to their similarities to humans in terms of anatomy, physiology and biochemistry. Indeed, many animals suffer from afflictions that also affect humans e.g. tuberculosis, cancers, and asthma[5]. Importantly, the pathogenic mechanisms associated with these diseases in humans and certain animals are very similar, so that 90% of veterinary drugs used to treat numerous conditions such as hypertension, cancer, and epilepsy in animals are very similar to those used in humans[14]. Most aptly, a recent study investigating the transmission route of SARS-CoV-2 using ferrets found that they present with an upper respiratory tract infection and long-term

viral shedding similar to that seen in humans[13,15]. Additionally, non-human primates have been shown to develop immunity against SARS-CoV-2, thus offering an unparalleled model for its study[13,16]. Moreover, animals have short life-cycles and can thus be studied throughout the course of their lifetimes or even across generations, and their environment can also be easily controlled and regulated (e.g. in terms of diet).

These factors have made animals pivotal in advancing our scientific knowledge of basic biological processes[17]. The resulting understanding has, in turn, been fundamental to treating a wide range of human diseases using drugs, vaccines, and other therapies, as well for toxicology testing. For example, animal testing was crucial in the development of the smallpox vaccine, a virus that killed 300-500 million people before its eradication in 1980[18]. More recently, work in ferrets and non-human primates has contributed to the development of the ChAdOx1 nCoV-19 vaccine which, together with other vaccines, is critical for the treatment of COVID-19[19]. This intermediate step is essential, as illustrated by a vaccination study in ferrets against SARS-CoV-1 in 2004, in which some vaccinated ferrets developed hepatitis instead of immunity[20]. Clearly, this level of risk would not be tolerated in trials on humans, especially given the already slow vaccine uptake in many countries.

It is important to remember that scientific research does not start with animal testing. The biological complexity of humans and other mammals is based on intricate networks of molecules and cells. Basic research focuses initially on these interactions, through a range of *in vitro* and modelling techniques which provide the basis for further studies in living tissues[21]. However, a complete understanding of the human body requires integration of knowledge at the molecular, cellular and higher organisational levels. Animal experimentation plays an important intermediary role in the translation of such fundamental studies into medical applications[17].

Historically, the development of important therapies and preventative treatments has relied heavily on animal research. Examples include antibiotics, insulin, vaccines, and cancer treatments. Tumour studies in mice using humanised cell lines enable the identification of effective chemo-immunotherapies that are subsequently subjected to clinical trials for possible approval and distribution for patient benefit[22]. Without the existence of appropriate mouse models and humanised cancer cell lines, these discoveries would be almost impossible to achieve: the high risk of side effects or even worsened prognoses demands strict regulation and criteria which animal testing is central to meeting. Another example is the development of Highly Active Anti-Retroviral Therapies which have extended the lives of patients with AIDS to near normal levels[23], a feat deemed impossible just a few decades ago. Additionally, modern surgical techniques have been perfected in animals and many people now regularly benefit from kidney and heart transplants, hip replacement therapy, and life-saving blood transfusions[5].

In conclusion, it is apparent that animals are both excellent models for the study of human biological processes and also critical for studying the efficacy and safety of therapies and other interventions, thus enabling scientific and medical advancements[7].

Limitations of animal research

However, there are also limitations to animal research. The procedures used are very varied in terms of severity and the types of animals used. These procedures can be as benign as observing the animals in their own natural environments, or as extreme as causing significant pain and ultimately death. Unfortunately, most animals in labs are killed once the experiments are finished and this often happens after a number of invasive techniques are performed. Many of these involve the administration of drugs and/or extraction of blood which can cause substantial discomfort and distress. Additionally, differences in physiology and metabolism between species lead to mismatches in the absorption of these drugs/products, so that over 90% of tested compounds do not meet the

safety standards in humans by the FDA[24]. Moreover, the shorter lifespan of animal models can make it difficult to assess the long-term effects of the tested substances. Further, animal tests are expensive and time-consuming, and non-standardised regulation across different countries often results in repetition, greatly increasing the cost of animal testing, both in terms of money and loss of life[25].

In the UK, the Home Office strictly monitors the number of scientific procedures conducted on living animals. In 2019 this number was approximately 3.4million[26]. 51% of these were experimental procedures, and 93% involved mice, fish or rats [Fig 1][27]. 57% of all experimental procedures were for the purpose of basic research, primarily targeting the nervous system, the immune system and, unsurprisingly, cancer. These figures may seem significant, but when compared to the numbers of animal deaths caused by other factors (most notably agriculture), even when ignoring the huge and widespread benefits that animal research brings to humanity, they are extremely small [Fig 2][27].

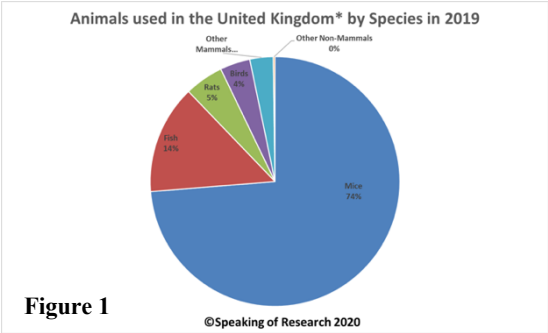

Figure 1

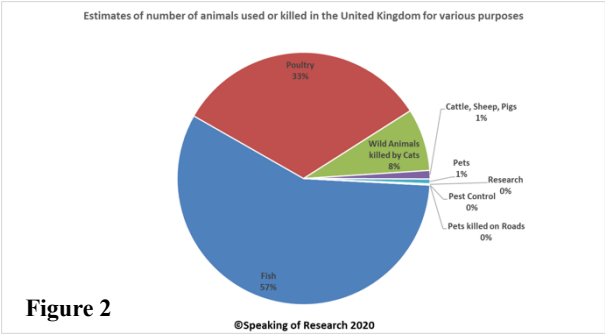

Figure 2

Conclusions and future considerations

While it is clear that the scientific community is committed to reducing, replacing, and refining animal research through the development of new technologies and techniques, the overriding consensus remains that animal research is fundamental for scientific progress and thus advancement in medical care and patient benefit. This is particularly true for the aspects of research that cannot currently be conducted by any other means. Therefore, until/unless feasible alternatives are developed, animal research will remain a central part of the scientific process for as long as is necessary. This should not be a cause for despair, but rather a scientific triumph that should be celebrated. Without animal research modern medicine would not exist, life expectancy would not be so greatly increased, and illnesses that once stole the lives of so many would not be treatable and managed by antibiotics, vaccines, and surgical procedures.

Rather than questioning the purpose and role that animal research plays in society, it seems more constructive to focus future efforts on the following: 1. Review and uphold regulation that protects animals both nationally and internationally, without implementing unnecessarily bureaucratic barriers that harm science more than they protect animals[13]. 2. Continued engagement and education with the public and policy makers by scientists, to provide additional clarity and explanation where this may currently be lacking. Surprisingly, the use of animals in research is considered a much greater infringement on animal rights than their use in food[27]. This is a clear contradiction and arguably a hypocritical and flawed assessment of animal rights. As discussed, experimental animals are regularly and closely monitored to minimise suffering and maximise wellbeing, the involved scientists are rigorously trained, and only procedures that have been justified and approved by the Home Office are conducted. Only a tiny percentage (approx. 5-7%) of animals undergo procedures deemed “severe” or “non-recovery”[27], and only 3 million animals are used for these procedures per year. This is in contrast to the 6.4 billion land and sea animals that are killed p.a. to support the UK food supply, the majority of which suffer abhorrent living conditions. Yet, only 3% of the UK population object to this by eating a plant-based diet, whereas over 40% of the UK public object to the use animals in research[2], despite the fact that their use in this context is so much more important to humanity.

Therefore, moving forward, it is essential that these widespread biases, perceptions and misunderstandings are deconstructed. 2/3 of the UK public feel insufficiently informed about the use of animals in research[2]; presenting an opportunity for improved education and engagement that cannot be missed. The public are utterly reliant on scientific research, but the scientific community is also reliant on public support, trust, and engagement. This co-dependent relationship has perhaps never been more obvious than during the COVID-19 pandemic. A transparent and accessible discussion is needed so that, whilst animal research remains as ethical and effective as possible, scientific progress and medical advancement remain an unwavering priority forevermore.

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