

THE ETHICAL JUSTIFICATION FOR THE USE OF ANIMALS IN BIOMEDICAL RESEARCH

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Abstract - Despite all the benefits, the use of animals in biomedical research is still a subject of debate with respect to its true value. The sensitivity of the community and the interest of scientists who work in the field of laboratory animal science and welfare have clearly demonstrated that the use of animals in biomedical research must be conducted under specific scientific, legal and ethical rules. The ethical justification of a research project starts from its initial designing phase until its completion and the review of the obtained results. Justification of the necessity of the project and the need to use animals in the interests of human or animal health, the importance of conducting a pilot study and a systematic review of previously published animal research on the topic, and the availability of the proper facilities, equipment and personnel are the main issues of concern in the ethical review of a research project. The ethical justification of the proposed project by the scientists themselves involves team-work, and should be a sustainable rather than a one-off procedure. This justification reflects the interest and the responsibility of scientists to reduce the number of animals, refine the procedures, and possibly replace animals in their research projects. The end-results of the ethical review process will be the creation of a trust relationship between scientists and society.

Keywords: Laboratory animals, ethics, refinement, reduction, replacement, animal experimentation

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INTRODUCTION

The use of animals in biomedical research has always played an important role in the acquisition of scientific knowledge for understanding basic biological processes and for improving the quality of life of humans and animals. Recently, the development and use of genetically modified animals have also given scientists the opportunity to better study the role of specific genes, and this has created new perspectives in combating and treating diseases such as gene therapy. Despite all the benefits, the use of animals in biomedical research continues to be a subject of debate with respect to its true value. Opponents on the use of animals in any kind of research believe that animal experimentation should be abolished immediately (Festing and Wilkinson, 2007). On the other hand, there are legal requirements that oblige scientists who conduct animal-based research to ensure that their experiments are conducted humanely in order to

satisfy societal concerns and to ensure the quality of their experimental results (European Council, 1986).

Nowadays, animal experimentation is considered an ethical issue (Nuffield Council on Bioethics, 2005; Rollin, 2007). A variety of different ethical review processes that are based mainly on different legal systems and cultural backgrounds have been established around the world. The aim of these ethical reviews is to ensure that the 3R's concept of Russell and Burch for the replacement of animals to be used in the research project, reducing the number of animals required for the research project, and refinement of the experimental procedures, are respected (Russell and Burch, 1959). Although there are no legal requirements in the existing European Union (EU) legislation, compulsory ethical review of all scientific uses of animals exists in sixteen of the twenty EU countries which are members of the Federation of European Laboratory Animal Science Associations (FELASA)

(Smith *et al.*, 2007). This ethical review is done by institutional, local, or national committees. Furthermore, it is within the scope of the revised EU Directive 86/609 to define and harmonize the ethical review process within the member states of the EU (European Commission, 2008). The application guidelines of the Seventh Framework Program of the EU require the ethical documentation of all projects that propose the use of animals. This requirement clearly demonstrates that the EU is very concerned about the ethical use of animals in EU-funded scientific projects. From this perspective, scientists in the EU have a moral obligation to ethically justify the use of animals in any animal-based research project.

ETHICAL JUSTIFICATION

For many years, scientists justified the use of animals in their experiments solely by referring to their scientific value in human health and welfare. Aside from a purely anthropocentric perspective, ethical concerns about the compromised animal welfare in animal experimentation cannot be tempered by human benefits alone (Olsson *et al.*, 2007). It is equally important that the benefits of the experiment are achieved with minimal negative consequences to the animals.

No matter which process of ethical review is applied, it is crucial that scientists realize the importance of ethics in their animal-based research. Scientists themselves should be aware of the need to conduct an ethical justification of their projects. Moreover, they should conduct ethical justification of their project from its initial design until its completion and review of the experimental results. It is also the responsibility of scientists to acquire knowledge about the locally existing laboratory animal welfare legislation and to conform to the letter and spirit of these laws.

The main objective of the ethical justification is to ensure that the project is scientifically and ethically justified as required by law. Moreover, the process should ensure that the decision to use

animals for research was made after earnest consideration of its specific animal welfare issues, and that the project is designed in such a manner that it will be conducted humanely and be environmentally-friendly. The ethical justification of a research project by scientists themselves involves teamwork, and should be sustainable rather than being a one-off procedure. In order for the process to be effective, the ethical justification of a project by a research team should be supportive and result from an open discussion and exchange of opinions among its members. For this purpose, the active collaboration between scientists with different background knowledge within the research group is necessary. A biostatistician can assist the researchers in obtaining valid statistical results from the minimum possible number of animals. A laboratory animal veterinarian can contribute to the refinement of the experimental techniques by suggesting the most appropriate anesthetic and analgesic protocol for the experimental procedure, the determination of a humane experimental endpoint, and the method of euthanasia. It is very important that a layperson be involved in discussions about the aims of the project. This person represents the community, and can convey societal concerns to the scientists about the proposed project. Ethical justification should ensure also that the humane care and use of the animals will take place from the beginning to the end of the animal experiment.

Justification of the necessity of the project

The primary concern of the research team is to justify the necessity of the project itself, the likelihood of its success, and, of course, the scientific significance of the project for the benefit of human or animal health. To this end, researchers should state clearly: (a) the objectives of the research project and/or the hypotheses to be tested, (b) the reason for choosing a particular animal model, (c) the animal species and strains to be used, as well as the supply sources, (d) the details of each separate experiment that will be conducted in the research project, (e) the study design and the

number of animals to be used, and (f) the statistical methods that will be used to analyze the experimental results.

Justification of the need of the research project, clarification of its goals, and its predicted scientific benefits are based mainly on the amount of scientific knowledge of the research team. This information should be presented in the application by providing the relevant background information and bibliography and highlighting the unresolved problems. This avoids unnecessary duplication of research, although duplication may be required in pilot studies that involve a novel method. The research team should also clearly define their expectations of the research project, and in what way this new information will increase the existing scientific knowledge and/or benefit the health or welfare of humans or animals. The social impact of the project should be stated also. Finally, the scientific purpose of the research project should be of sufficient substance in order to justify the use of animals.

Justification of the use of animals

Prior to any animal use, a cost-benefit analysis should be done in order to determine whether the obtained benefits of the animal-based research will outweigh the cost to the animal(s). The term "cost" defines the expected harm, pain and distress that is likely to be experienced by the animals during the conduct of the research project. The term "benefit" defines the potential benefits of research that might be realized for humans, other animal species, or the environment. The use of animals in a research project is only acceptable when its expected benefits will outweigh the physiological and psychological harm that will be caused to the animals. When preparing a research protocol, special concern should be given to the minimization of pain, distress and discomfort, and the maximization of possible benefits.

For many years, the 3R's concept of Russell and Burch on the reduction, refinement and replacement of animals has become an integral component of the design and the realization phases of an animal experiment (Russell and Burch, 1959;

Guhad, 2005). Replacement refers to methods which avoid or replace the use of animals in a research project where animals would otherwise have been used. This includes both absolute replacement of animals with systems or methods, such as *in vitro* methods and computer programs, and relative replacement such as replacing sentient animals, such as vertebrates, with less sentient animals such as some invertebrates that, according to current scientific evidence, have a significantly lower potential for pain perception (Wakefield et al., 2002). The research team should provide all the necessary documentation of the replacement methods that were considered during their planning discussions of the research project. In some cases, the replacement methods could contribute to the reduction of the number of animals that will be used in the research project. *In vitro* or *in silico* techniques could be used, for example, to monitor the influence of nanomaterials on the cardiovascular system and circulating blood.

Reduction refers to any strategy that will result in sufficient data to answer the research question by using the smallest number of animals in order to obtain valid experimental results, or by maximizing the information obtained per animal. In this way, subsequent use of additional animals and compromising animal welfare can be avoided or potentially limited. The number of animals to be used in a research project can be minimized by several means. Scientists should know what has been done previously by other scientific teams in the chosen area of research and use this information to estimate the number of animals needed in order to produce reliable results. The number of animals to be used in the research project can be reduced also by good experimental design and careful observation so that several variables can be analyzed simultaneously (McConway, 1992; Chiarotti and Puopolo, 2000; Nevalainen, 2004). The research team should also keep in mind that using too many animals can lead to the unnecessary use of animals, and using too few animals may require repetition of the entire experiment because the results may be unreliable after analysis of the data. In both

instances, there is wastage of animals and other resources (Festing and Altman, 2002; Festing, 2006).

Refinement refers to any modification of animal husbandry or experimental procedure that will result in the minimization of pain and distress and in the enhancement of the welfare of an experimental animal during its entire life. Refinement is a process that begins with a critical evaluation of the experimental intervention. It also encompasses an assessment of animal welfare, the recognition of poor or suboptimal welfare and identification of the causes, the selection of welfare improvement strategies, and the implementation of one, some, or all of these strategies. Any changes then have to be evaluated for their efficacy. In doing so, a refinement loop can be created because the process begins again (Lloyd et al., 2008).

Housing animals under minimal conditions by providing, for example, only food, water and bedding, are likely to cause some distress to the animal even before any experimental procedures have been initiated. Refinement of housing conditions by environmental enrichment (social or physical) could minimize the discomfort of the animals (Baumans *et al.*, 2006; Kostomitsopoulos *et al.*, 2007). If housing animals under minimal conditions is necessary, it should be ethically justified.

Special concern should be also given to procedures that minimize pain, suffering and distress. For any project that might involve pain, suffering or distress, the research team should assess thoroughly whether the information that is to be gained can be justified, and explore whether non-animal alternatives can be used. Any procedure that is likely to cause pain should only be performed under adequate local or general anesthesia and with appropriate analgesia, unless anesthesia and/or analgesia compromise the experimental aims (Bateson, 1991). If it is decided that either anesthesia or analgesia are not to be used to alleviate pain, this should be ethically justified. Special concern should be given also to the recognition of an early humane experimental endpoint, and the method of euthanasia of the

animals which should be performed by trained personnel only (Morton and Griffiths, 1985).

The ethical justification should include an assessment of the estimated pain, and the severity of the procedure should be classified (Smith et al., 2008). A retrospective analysis is recommended also in order to compare the intensity of actual and predicted pain in the experimental animals (Jennings *et al.*, 2005).

Special justification should be provided when creating and using genetically modified animals. Special attention should be shown to these animals whose health and/or welfare may be compromised because of gene modification(s) (van der Meer *et al.*, 2001; Wells et al., 2006).

The pilot study

A pilot or feasibility study is a small experiment that is designed to test the logistics of the proposed research project, and to gather this information prior to a larger study. Therefore, it is conducted in order to improve the quality and efficiency of the project. A pilot study can reveal deficiencies in the design of a proposed experiment or procedure which can then be addressed before time and resources are expended in the large-scale study. For an animal-based experiment, a pilot study can provide useful information on the estimated number of animals that are needed for the study, as well as information on the severity of proposed procedures or treatment. When conducting a pilot study, the research team will have the opportunity to further refine the experimental procedure(s) by using different anesthetic or analgesic protocols or advancing the humane endpoint to an earlier time point. Lastly, pilot studies should use a small number of animals, and should always be approved by an ethical review panel beforehand (National Centre for the Replacement; Morton, 2008).

Systematic review of animal research

The systematic review is a synthesis of all available research literature by addressing a specific research

question using a systematic approach. Systematic reviews involve: (a) a planned, rigorous, clearly defined and explicitly documented methodology, (b) a specific search of bibliographic databases to identify all relevant studies, (c) appraising and selecting studies for the review using defined criteria, (d) analyzing the experimental methods of all the included studies to assess the quality of the study design and conduct, and (e) assessing the results of the included studies (National Centre for the Replacement). Part of the systematic review is meta-analysis which is a statistical technique for combining the numerical results from the independent studies that were reviewed in the systematic review (Deeks et al., 2001). The meta-analysis result increases statistical precision. It represents an overall and more reliable estimate of the treatment effectiveness of healthcare interventions than the results of an individual study alone.

Systematic review and meta-analysis have a positive impact when considering the 3R's. A systematic review could result in reducing the number of the animals because duplicate animal experiments would not be performed. Meta-analysis of the results of previously published similar experiments will allow a more precise estimation of the number of animals that need to be used in the proposed research project. Systematic reviews of comparative studies could be used to provide evidence of the effectiveness of refinements. They could also be used to provide evidence of the effectiveness and validity of *in vitro* studies, the use of invertebrates, or *in silico* data with traditional animal models such as when used for testing the efficacy of a particular drug (National Centre for the Replacement).

Facilities, equipment and personnel

The research team should justify the availability of the appropriate experimental facilities, equipment, and personnel that are required to conduct the animal experimentation. The housing conditions and facilities for experimental animals must comply with existing national or international legislative requirements or guidelines that relate to animal health and welfare (National Research Council,

1996; European Commission, 2007). Depending on the animal species, researchers should consider not only the minimum housing and management requirements that are set out in legislation, but should also refer to the most recently published literature on these topics in order to consider improvements to the housing and management of their experimental animals. All the necessary equipment for the realization of the animal experiment should be available to ensure the quality of the experiment.

It is imperative that the people who work with experimental animals are fully trained in animal care and understand the needs of the species they are caring for, as well as the experimental procedures that are to be used in the research project. This includes the ability to observe and assess when an animal may be in pain or distressed, and the knowledge to then take the relevant measures to effectively alleviate pain or distress as quickly as possible. Animal carers should be fully aware of the legislation that is relevant to the housing and management of experimental animals, and their use in a particular study. The researcher should ensure that all personnel are responsible, trustworthy and have the relevant experience to perform their specific duties within the study. Education and training guidelines are available from numerous international organizations and scientific associations, such as the Council of Europe, the World Health Organization, and FELASA (Council of Europe, 1993; Nevalainen Convenor et al., 2002).

CONCLUSIONS

Despite the fact that many alternative methods for replacing animals exist, the use of animals in biomedical research is still unavoidable. The public expects high standards of care and use to be applied to research animals. Legal requirements oblige the scientists who conduct animal-based research to ensure that their experiments are conducted humanely in order to satisfy societal concerns, and to ensure the quality of their experimental results.

These clearly define the moral obligation of the research team to ethically justify the animal experimentation to the ethical review panel and to the community. The ethical justification of laboratory animal research protocols reflects the interest and the responsibility of scientists to reduce the number of animals, refine the procedures and possibly replace animals in their research projects. This process will build a trust relationship between scientists and society. It is also very important to educate the next generation of scientists to think about both ethics and science. As animal welfare goes, there should be no doubt that ethics must go hand-in-hand with good science in order to further improve animal-based biomedical research.

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