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Chapter 9

Alternatives to Animal Experimentation: Its Institutional Teaching and Scientific

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Abstract

Although it is desirable to replace scientific procedures with live animals by other methods that do not use them, the use of animals in scientific procedures should be restricted to those areas that benefit human, animal, and environmental health. The use of animals as experimental models of observation of biological phenomena has evolved with man, to this day. The use of animals for scientific or educational purposes should be considered only when there is no other alternative and it is governed by the principles of replacement, reduction, and refinement. The scientists should be sure that the information obtainable with the experiments is not yet available or that the protocol was designed taking into account animal protection considerations. The chosen methods must use the least number of animals; provide satisfactory results; use the species with the least ability to experience pain, suffering, anguish, and damage; and be optimal for the extrapolation of results to the target species such as humans. It will be fundamental to guarantee on a scientific and ethical basis that the use of an animal is subject to a careful evaluation regarding the scientific or educational validity.

Keywords: animal experimentation, animal model, laboratory animals, research design, animal testing alternatives, animal

1. Introduction

The research is focused today on the ethical, logistical, economic, scientific, and legal requirements. At the European level, Directive 2010/63 of the European Parliament and of

the Council of September 22, 2010, on the protection of animals used for scientific purposes [1] must be highlighted, which is translated into Spanish legislation by Royal Decree 53/2013 [1, 2]. Researchers have to demonstrate the real need to use animals in scientific and teaching applications. These regulations aim to ensure animal protection and, in particular, adequate care for animals; not unnecessarily cause pain, suffering, anguish or prolonged injury; avoid duplication of procedures; minimize the number of animals used in procedures; and apply possible alternative methods.

Russell and Burch [3] formulated for the first time the "principle of the three Rs" that was adopted by the aforementioned regulations. Russell and Burch considered that the replacement was the ultimate goal of the investigation. Its main message is, in summary, that, if we are to use a criterion to choose which experiments to carry out, that of humanity is the best we can ever conceive and that the greatest scientific achievements have always been the most human and the most esthetically attractive, those that best transmit that sense of beauty and elegance that constitutes the very essence of science in its best aspect. Animals should be replaced by less sentient alternatives such as invertebrates or *in vitro* methods whenever possible. Only an experiment with live animals should be carried out if there is no alternative method for the procedure we wish to perform (replace), for example, using audiovisual media or virtual reality techniques [4].

Secondly, if the alternative method does not exist and we have to perform the experiment with live animals, the number of animals should be reduced to the minimum consistent with the scientific objectives of the study, recognizing that important biological effects may be missed if too few animals are used [4].

And thirdly, we are also told that we must modify the procedures used so that animals suffer as little as possible (refine). Experimental protocols should be refined to minimize any adverse effects for each individual animal. For example, appropriate anesthesia and analgesia should be used for any surgical intervention. Death is not an acceptable endpoint if it is preceded by some hours of acute distress, and humane endpoints should be used whenever possible. Staff should be well trained, and housing should be of a high standard with appropriate environmental enrichment. Animals should be protected from pathogens [4].

Its main message is, in summary, that, if we are to use a criterion to choose which experiments to carry out, that of humanity is the best we can ever conceive and that the greatest scientific achievements have always been the most human and the most esthetically attractive, those that best transmit that sense of beauty and elegance that constitutes the very essence of science in its best aspect. Royal Decree 53/2013 aims to establish the applicable standards for the protection of animals used, bred, or supplied for the purpose of experimentation and other scientific purposes, including education and teaching. For this, it regulates the following:

1. Basic investigation
2. The application of the scientific method in which a problem is first identified and observations, or other relevant data are then used to construct a solution:
3. Prevention: prophylaxis, diagnosis or treatment of diseases, or their effects on humans, animals, or plants

4. Evaluation: detection, regulation, or modification of physiological conditions in humans, animals, or plants
5. The welfare of animals, particularly the improvement of the conditions of production of animals
6. Evaluate the efficacy and safety of new pharmaceutical products
7. Research directed to the conservation of the species
8. Protection of the natural environment in favor of the welfare of human beings or animals
9. Higher education or training for the acquisition or improvement of professional skills
10. Legal and forensic medicine

The use of animals in scientific experiments likely to cause pain, distress, or lasting harm generates important ethical issues. Animals should be used only if the scientific objectives are valid, there is no other alternative, and the cost to the animals is not excessive. "Validity" in this case implies that the experiment has a high probability of meeting the stated objectives, and these objectives have a reasonable chance of contributing to human or animal welfare, possibly in the long term [4].

Scientists who use animals in research must justify the number of animals to be used, and committees that review proposals to use animals must review this justification to ensure the appropriateness of the number of animals to be used. Obtaining satisfactory scientific results, it will depends of sample size calculation should be performed as well as, the election of more suitable animals [5].

2. Criteria for the evaluation of a project

Regulation D2010/63/EU aims to establish the applicable standards for the protection of animals used, for scientific purposes [6]. It establishes for the first time in EU legislation the principle of "the three Rs" and imposes it as a firm legal requirement in all aspects of the care and use of animals in that area. The directive, in its application, goes beyond the initial interpretation and also regulates the breeding and care of the animals, that is, guarantees refinement during housing, breeding, and care, even if the animal is not object of any scientific procedure, regulating the following:

1. The experimental protocol should be with respect to the project objectives.
2. The use of animals for scientific or teaching purposes should be considered only when there is no alternative.
3. The objectives cannot be achieved by alternative methods.
4. Ethical considerations in the use of animals are the basis of the authorization of projects.

5. The application of the principles of replacement, reduction, and refinement must be guaranteed.
6. The means are put in place so that the animals do not necessarily suffer, and they are provided with analgesics and anesthetics to minimize the suffering or anguish.
7. Euthanasia methods appropriate to the animal species and the procedure performed are used.
8. The personnel participating in the procedures have the appropriate training (training and experience) to carry out the tasks entrusted to them.
9. The procedures are classified according to their degree of severity.

An experiment is a procedure for collecting scientific data in a systematic way to answer a question correctly or for the generation of new hypotheses. All research should be described in such a way that the study design could be repeated elsewhere [1, 2] (**Table 1).**

Animal research has made major contributions to the health and welfare of humans and domestic animals. These and many other advances have enabled physicians to treat a wide range of human diseases. Many experiments appear to be poorly designed and inadequately analyzed and reported. As a result, some are found to be unrepeatable, leading to a waste of animals and scientific resources. Critical appraisal is an essential part of the scientific process designed to assess the validity of scientific findings. The new techniques of systematic reviews and meta-analyses are hampered by poorly written papers. The importance of randomization and blinding does not always seem to be understood, and it seems that many scientists have inadequate training in experimental design and statistics [7]. Animal studies differ from clinical studies in some aspects, such as the diversity of animal species studied, experimental design, and study characteristics. These methods used in animal studies are explained in [8]. Systematic reviews "can help improve the methodological quality of animal experiments, make the choice of an animal model and the translation of animal data to the clinic more evidence-based and implement the 3Rs," according to [9].

(1). The objectives of the research and/or the hypotheses to be tested
(2). The reason for choosing their particular animal model
(3). The species, strain, source, and type of animal used
(4). The details of each separate experiment being reported, including the study design and the number of animals used and
(5). The statistical methods used for analysis.
(6). Accommodation conditions, for the care of animals
(7). Euthanasia methods
(8). As well as training of the people who participated in the project

Table 1. Considerations in the study design.

3. Importance of animal experimentation

For ethical and economic reasons, it is important to design animal experiments well, to analyze the data correctly, and to use the minimum number of animals necessary to achieve the scientific objectives—but not so few as to miss biologically important effects or require unnecessary repetition of experiments [4]. The 3Rs—replacement, reduction, and refinement—can be applied to any animal experiment by researchers and other bodies seeking to conduct those studies in as humane manner as possible. Key to the success of this endeavor is an appreciation of the principles of good experimental design and analysis; these need to be considered in concert before any data is collected and understanding of animal welfare plays a central role in laboratory practice—are to the betterment of research per se [40]. Careful choice of the animal model is essential, if research is to be conducted efficiently, by using the minimum number of animals in order to provide the maximum amount of information. Inbred strains of rodents provide an excellent way of controlling and investigating genetic variation in characters of interest and in response to experimental treatments. Outbred stocks, in which genetic and nongenetic factors are inextricably mixed, are much less suitable, because random and uncontrolled genetic variation tends to obscure any treatment responses [10].

There is concern about the lack of repeatability of many preclinical experiments involving animal toxicity tests in rodents used to assess the safety of drugs to detect adverse effects that have not been formally evaluated. However, the test does not specify the strain of animals in which the genetic variation, is unknown and uncontrolled; a better strategy would be to use small numbers of animals of several genetically defined strains of mice or rats instead of the undefined animals used in the present. Inbred strains are more stable providing more repeatable data than outbred stocks [11].

4. Choice of animal model

One of the uses of animal models is related to the evaluation of new drugs for the treatment of human diseases. For this type of use, the animal model must respond adequately to the effects of different therapeutic agents. The failure rate of investigative new drugs is excessively high, ranging from about 80 to 97% depending on the therapeutic area. Some of this may be due to poor design of the animal studies. But in some cases, the animal model may not be truly representing the human condition. It is suggested that a good model of a human disease should also have the same human biomarkers of that disease [7]. Compounds that are active in routine clinical practice should show activity in the model (positive controls), and compounds that show no activity in clinical practice should not show effects in the animal model (negative controls) [12].

4.1. Classification of animal models

Most animal laboratory models have been developed and used for the study of the cause, nature, and cure of diseases in humans. There are five categories of experimental models, of which the first three are the important ones, since they are the most used:

a. Induced animal models

b. Models generated by genetic modification

c. Spontaneous animal models

d. Negative animal models

e. Orphan animal models

The selection of any animal model for research should be based on the following considerations: models based on analogy (similar structures involve similar functions) and models based on homology (structures derived from the same evolutionary precursor have the same or similar functions). The most appropriate selection of an animal species for the experimental purpose should not be based on its easy management due to its small size, availability, familiarity, or cost [13].

However, scientists recognize that there are no real substitutes in the use of laboratory animals. Studies with bacteria, tissue cultures, and computer simulations can provide useful information, but the complexity of living organisms requires research and analysis on animals similar to humans to achieve reliable results. When considering which can be the best animal model to use, it is important to take into account the extrapolation or generalization of results that this model generates. For example, in neuroscience it simplifies the results obtained between models in a simplified way [41]:

a. Homologous models: causes and symptoms are identical animal/man. It is only possible in the case that in the animal model, the respective injuries to the associated syndromes resemble each other.

b. Isomorphic models: similar symptoms but the cause does not have to be the same. For example, in a neural zone degeneration pathology, we can alter that same area in rat brain and see that the symptoms are identical.

c. Partial: Some of the models do not completely imitate the human disease, but they can be used in the study of certain aspects or treatments of the human disease, considering that an optimal model would be one that develops a comparable symptomatology, etiology, and neurophysiological background and that responds similarly to the effects of different therapeutic agents.

5. Practical aspects of experimental design in animal research

To designing any scientific investigation once, having an idea for a research project is necessary to make a review of the literature and to get the information that is necessary for the experimental design phase. A null and an alternate hypotheses that address the problem statement are then formulated, and only then is the specific design of the experiment developed. The identification of the most appropriate animal model to address the experimental

question being asked is very important. Other aspects are the considerations that include the number of animals needed per group and evaluating the most appropriate statistical analyses [14]. Nowadays models of human diseases are necessary for experimental research into the biological basis of disease and for the development of treatments. They have an enormous impact upon the success of biomedical research. However, in spite of this, a consistent system for evaluating, expressing, and comparing the clinical validity of disease models is not available [14].

Usually, studies are performed on animal species such as genetically heterogeneous (GH) mice, and rats continue to be used in research even though the case for using isogenic strains has been argued repeatedly. GH stocks represent poor material for controlled studies because genetic heterogeneity normally leads to phenotypic variability and a decline in experimental sensitivity. Isogenic strains are a vital, proven, and powerful resource for biomedical research and should be used in preference to GH stocks by all scientists who use laboratory rodents [15].

It is impossible to give specific rules for the selection of the best animal model; however, it is convenient to make many considerations before an experiment. These are some general rules regarding the criteria for choosing the model [16] **(Table 2**).

It is also important to identify in usual practice among other criteria for the selection or rejection of a model the presence of diseases or special conditions of the animal and that the microbiological status of animal can influence their response [13]. These factors should be

(1). Suitable as analogous
(2). Ability to transfer information
(3). Genetic uniformity of the organisms used
(4). Knowledge of biological properties
(5). Cost and availability
(6). Generalization of results
(7). Ease and adaptability to experimental manipulation
(8). Ecological consequences
(9). Ethical implications
(10). Availability of accommodation
(11). Size of the animal
(12). Number of individuals needed
(13). Life expectancy
(14). Sex
(15). Amount of data needed
(16). Age of animals
(17). Need of offspring

Table 2. Alternative procedures in teaching and training.

considered when choosing the animal model that best suits the experimental purpose. Many models that do not use animals have also been developed, refined, and characterized. These models are useful in some types of research and testing, and they can often be used to complement work with live animals.

6. Alternative procedures in education and training

Animals have been used in research and teaching for a long time. However, ethical guidelines and pertinent legislation were instated only in the past few decades; even in developed countries guidelines for animal experimentation vary. With the advent of newer methodologies in human cell culturing, novel/emerging methods aim to minimize, if not avoid, the usage of animals in experimentation [17]. The European Partnership for Alternative Approaches to Animal Testing (EPAA) activities are focused on international cooperation toward alternative methods. The EPAA is one of the leading organizations in Europe for the promotion of alternative approaches to animal testing [18].The alternative methods are based on the principle of the 3Rs [19] established by Russell and Burch in 1959: R of reduction, using only the number of animals needed to obtain a reliable and accurate information; R for refinement understood as any system that allows to reduce the severity of the damage inflicted on the animals; and R for the replacement of vertebrates by any other method that uses nonsensitive material. All methods or techniques that could substitute the experiments carried out with animals, reduce the number of animals used in each trial, or improve existing procedures in order to reduce stress and avoid the suffering of the animals that are included. The principle of the 3Rs has been responsible, in large part, for the drastic reductions in the use of laboratory animals that have occurred in the last century and for the significant changes in the techniques of research, testing, and education for the benefit of science and public health, as well as animals.

Undoubtedly, the promotion of alternative approaches is one of the basic aspects that permeate the new animal protection regulations. This is the terminology used in Directive 2010/63/EU and consequently in Royal Decree 53/2013 [1, 2]. Experimental alternative methods include any procedure that replaces the use of animals, that reduces the need for animals in a particular test, or that refines a technique in order to reduce the amount of suffering endured by the animal. To be used in the toxicity tests required for the register prior to the commercialization, transportation, and use of a new chemical compound, it is necessary for the experimental procedure to be accepted by regulatory authorities. Thus, after its development, the method has to fulfill the phases of prevalidation (previous interlaboratory assessment), validation of its reproducibility and relevance to *in vivo* toxicity (final interlaboratory assessment), and the independent assessment of the study by a panel of experts and the progression toward regulatory acceptance. Also there must be the acceptance by international regulatory authorities of the fixed-dose procedure *in vivo* as an alternative to the classical assay of the determination of the toxicity by the mean lethal dose (LD50) which are key points on the promotion of the validation and acceptance of *in vivo* and *in vitro* alternative methods [20]. The principles of good laboratory practice (GLP) are designed to help ensure the proper management and conduct of studies. GLP compliance demonstrates to regulatory authorities that studies were

undertaken in a manner which promotes confidence in the data and reporting. Formal validation of *in vitro* toxicity studies is being recommended as an interlaboratory activity. Study management of interlaboratory studies in compliance with GLP is discussed [21].

The alternative approaches undoubtedly provide alternatives available to animal research to raise awareness of viable and, at times, even better options outside of animal experimentation. Outside of the well-established alternatives to animal experimentation like tissue culture methods including primary/continuous/immortalized cell lines, explant cultures, and organ cultures, several recent strategies have been recently mooted to curtail animal experimentation and simultaneously (and surprisingly) improve efficacy of data-gathering, while alternatives to animal experimentation may reduce research dependence on animal (through replacement). They currently cannot replace animal testing altogether. This impossibility exists despite several ethical, political, and financial "incentives" to persevere in this direction. The extant alternatives serve to complement animal experimentation in current research [17].

6.1. Ideal learning endpoints

In this way utilizing a multiple-choice test at the end of a course, the course participants would be assessed for a "reasonable" comprehension of percentile scores or percentage cutoffs [17]:

1. The spectrum of ethical issues pertaining to animal experimentation
2. A scientist's ethical responsibilities
3. A practical application of Russell's and Burch's 3R principles [3]
4. Application submission procedure to the local animal ethics committee
5. Recognition and relief of distress and pain in experimental animals
6. Basic animal handling, anesthetization, blood collection, drug administration, and euthanasia

6.2. Classification of alternative methods in teaching

The development of alternative methods for teaching is not new, and so in the report of the meeting of experts in alternative methods in teaching, organized by ECVAM in 1999 [24, 39], several types of methods were already identified:

There are several modalities of alternatives that can be used in teaching [22–24] **(Table 3).**

If an adequate system is not located, the bibliographic databases could also be revised. In general systems, the terms "education, training, teach*, instruct*, mannequin, manikin, simulat*, video, virtual, cadaver, software, computer'', etc., can be used. There are also systems aimed at improving the preparation of people who handle experimental animals.

The mechanical models consist of reproductions of animals or organs that allow training in management techniques, administration, extraction, and surgery. The classical audiovisual systems were the first used to show the techniques of animal handling, to learn comparative anatomy and various specific techniques. From the initial films, they were

(1). Mechanical models
(2). Audiovisual systems: Movies, videos, CD-ROM,
(3). Computer simulations and virtual reality systems
(4). *In vitro* tests: Ex. With cell cultures
(5). Observation and field studies
(6). Waste materials from slaughterhouses
(7). Clinical practices: human and veterinary
(8). As well as training of the people who participated in the project

Table 3. Alternative procedures in teaching and training.

converted into videos and are currently produced in digital format, CD-ROM, DVD, or downloaded from the Internet. Computer simulations and virtual reality systems have made a fundamental breakthrough that allows the student's interaction, which greatly accelerates learning [22].

6.3. *In vitro* models

The most interesting "animal substitute" to buttress preclinical drug development is the organs on chips (OOC) [26]. The OOC looks promising as a pathophysiologically pertinent model of experimentation.

In vitro models of skin pathophysiology and drug testing have been around for some time. Pioneering testing of human skin equivalents (HSE) included EpiDerm [27] and full-thickness EpiDerm [28]. Presently, HSE models are used to demonstrate simple physiology, to analyze autoimmune (disorders to malignancies) [29, 30]. These models may be better than animal models because the skin samples are human-derived. Additionally, these tissue models are grown *in vitro* in a biochemical and physiological simulating human homeostatic conditions, and they use Russel and Burch's principle of replacement [3].

However, animal testing will still be required for the foreseeable future. For example, a bacterial toxin had effects which were different from that on cultured cells [31] than its *in vivo* effects in a live animal [32]. Similarly a tested drug, owing to a multitude of reasons, may work fine on an *in vitro* model, but may not work (or may work differently) on a live animal. Therefore, *in vitro* models will effectuate manifold prescreening processes prior to animal experimentation but may only serve partially in reduction. Furthermore, only *in vivo* animal models can account for complex and/or unknown biological systems and pathways that *in vitro* models cannot encompass. Another example was a study conducted performed in *in vitro* systems and zebrafish embryos as alternative models for reducing rodent use in assessments of immunological and oxidative stress responses to nanomaterials demonstrated that some nanomaterials (NMs) stimulate oxidative stress and inflammation, which may lead to adverse health effects. The development of strategies for NM hazard assessment that promotes to use alternative models and non-rodent is being an important point of investigation of inflammation, and oxidative stress could make nanotoxicology testing more ethical, relevant, and cost- and time-efficient [33].

6.4. Computer modeling in silico

Pathophysiological simulations have been using high-tech computer modeling programs (in silico modeling) [33, 34]. Toxicity screening [35] and fundamental pharmacokinetic can be done rapidly *in vitro* depending on specific in silico modeling program availability [36]. There are additional software-based techniques (quantitative structure-activity relationships or QSARs) [37] that utilize estimates of a molecule's hazard-inducing capacity, based on its similarity to existing molecules, and extant human physiology. However, such simulations generally focus on major aspects and tend to overlook smaller but equally (if not more) important aspects.

6.5. Research involving human volunteers

Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) pertaining to brain activity has been used of research involving human volunteers is broached. However, there are several other "human testing" investigative methods which have been used. A classic example is microdosing; microdosing is implicated to early drug development; the pharmacokinetic data are acquired in humans using safe sub-pharmacologic "doses of drug" [38]. We currently still require animals to devise and test the efficacy and safety of therapeutic approaches as in mortality or toxicity studies. On the other hand, microdosing cannot predict adverse reactions of drugs that may occur at therapeutic levels, which animal studies clearly can. Therefore, microdosing can only assist in partial reduction of animal use in research. The way in which society views the use of animals in university learning and teaching has changed dramatically in the last 30 years. Debate by teachers and animal welfare advocates about the pros and cons of using animals in learning and teaching is widespread in the published literature, nationally and internationally, but rarely gives the students a voice. A study demonstrated the perspectives on the use of animals in learning and teaching, using on a survey of students at three Australian universities. The biology students value the authenticity of such experiences, the consolidation of theoretical learning, and the chance to use multiple learning modes via hands-on experiences. In particular, students see the benefits of such experiences as improving their understanding of biological concepts and opportunities for future employment [39].

When was compared upper level undergraduate students' evaluations of psychology laboratories using live rats with their evaluations of using a virtual rat (Sniffy). Students reported that the live-rat labs were ethically acceptable and that working with live rats enhanced their learning to a greater extent than working with Sniffy. These results support the retention of laboratories using live rats in psychology courses [25].

7. Conclusions

Animals have been used in research and teaching for a long time pretending to simulate human biology. The principle of the three Rs enunciated by Russell and Burch 3Rs (replacement, reduction, and refinement) is currently the most used animal ethics compliance guidelines for animal experimentation. Research pertaining to the efficacy of institutional ethical reviewing of animal research is sparse. The institutional ethical reviewing may work better in countries (and circumstances) which are more developed, have better

funding for animal facilities, have lesser bureaucratic impediments, have simpler/more direct processes, and have flexible common/statutory law providing allowance for better reviewing and penalty implementation. An animal experimentacion as a teaching resource contributes to the process of teaching-learning in bioethics for undergraduate students or university students.

Conflict of interest

All coauthors declare no conflict of interest.

Nomenclature

Directiva 2010/63 UE

Real Decreto 53/2013

3Rs principles

Normativa D2010/63/EU

Guidelines (OECD TG 407 and 408)

The European Partnership for Alternative Approaches to Animal Testing (EPAA) Directiva 2010/63/EU

The principles of Good Laboratory Practice (GLP)

Nanomaterials (NMs)

European Directive 2010/63/EU

Positron emission tomography (PET)

Functional magnetic resonance imaging (fMRI)

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