Chapter 1. Ethical aspects in animal research

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Research ethics is based on a set of moral principles and procedures and aims for compliance according to the ethical codes and the professional deontology of the R&D staff.

The general moral guidelines for the in-vivo laboratory research are:

- A correct evaluation of the results obtained
- The balance between benefits and risks
- Assessment of the need to continue the experiment throughout testing, with a particular focus on environmental protection, the biosphere and biodiversity.

Animal research also has to respect some basic rules: rule of precaution and rule of double effect.

The use of animals in research and testing, especially in the investigation of experimental pain in conscious animals, is an important ethical and political issue. In 1986 as a response to the constantly increasing pressure from the European public, the European Council adopted the European Convention for the Protection of Vertebrates Used in Experiments and other Scientific Procedures. Later regulatory norms were imposed by the Directive 2010/63/EU. Romania also has two laws: 305/2006 and 9/2008. The main purpose of these laws and directives is to reduce the number of experiments performed
Experimental models in rodents

on animals and the general number of animals used for research. Thus, animal studies can only be performed if the research is done for the prevention, diagnosis or treatment of diseases, in the field of forensic investigations, in education, for the study of physiological and environmental mechanisms.

The principles of ethical research also forbid the followings:

a) concealment or removal of undesirable outcomes;
b) manufacturing of results;
c) replacement of the results with fictitious data;
d) deliberately distorted interpretation of the results and forming conclusions;
e) plagiarism of results of other authors or publications;
f) deliberately distorted presentation of the results of other researchers;
g) incorrect authorship of a work;
h) introduction of false information in requests for grants or funding;
i) non-disclosure of conflicts of interest;
j) embezzlement of research funds;
k) failure to record and store the results;
l) lack of awareness of the research team before the start of the project on: labor rights, responsibilities, co-authorship rights to research results, funding sources and associations;
m) breach of confidentiality rules;
n) publication of or financing the same results.

I. Justification of the Research

EU regulations require ethical approval for all animal research studies. Information provided in the ethical approval forms
includes the relevance and importance of the study, potential short-term and long-term benefits. The experiment protocol must include methods of anesthesia, analgesia or other pain control methods in order to decrease the possible pain, discomfort and stress suffered by the animal. Exceptions are made in cases where pain reduction methods interfere with the research results and require, to the extent possible, that the painful stimulus applied to the animal does not produce permanent physiological changes.

A practical method for the implementation of these principles is represented by The Three R’s, proposed by Russel and Burch in 1959 that classify “the humane techniques” for animal research. The concept of the Three R’s has become an internationally accepted standard applied by researchers when deciding if and how to use animals for research, in the design of the experiments.

The Three R’s represent the foundation for current day ethical standards and are as follows:

- **Replacement**, refers to use of an in-vitro method over an animal one, whenever possible, but still achieving the same scientific aim: absolute replacement; or, refers to the replacement of superior animals (e.g. primates or mammals) with lower animals on the phylogenetic scale – (e.g. inferior vertebrates or invertebrates) - relative replacements. Alternately, live animals may be replaced with non-animal models, such as dummies for an introduction to dissection, for teaching the animal or the human body, mechanical or computer models, audiovisual aids or in-vitro modeling.

Advantages of replacement include utilizing pre-existing
knowledge for teaching, applying known principles to new systems to look for similarities, and using less expensive animals or models to screen large numbers of agents for toxicity or mutagenicity.

Disadvantages of replacement mainly stem from the fact that any models are dependent on pre-existing information. In a system as complex as a live organism, all of the variables in physiology and pathology are not known. Thus, any research on new biological processes must utilize a living organism at some point.

- **Reduction**, meaning decreasing the number of animals used in a certain experiment to the lowest value that ensures statistical validity, or extracting more information from the same number of animals. Methods to achieve this include:
  - Performing pilot studies to determine some of the potential problems in an experiment before numerous animals are used;
  - Designing a study to use animals as their own controls;
  - Gathering a maximum amount of information from each animal, perhaps gathering data for more than one experiment concurrently;
  - Consulting with a statistician to use the minimum numbers of animals required to achieve significance for the experiment;
  - Minimizing variables such as disease, stress, diet, genetics, etc., that may affect experimental results;
  - Performing appropriate literature research and consulting with colleagues to ensure that experiments are not duplicated;
  - Using the appropriate species of animal so that useful data
is collected;
  o Replacement whenever possible.

- **Refinement**, refers to the modification of experimental procedures to enhance animal well-being and minimize or eliminate pain and distress, thereby improving the quality of life for the animals and the quality of science;

A research on animals may not be conducted until the protocol has been reviewed by an appropriate animal care committee; typically an Institutional Animal Care and Use Committee (IACUC) to ensure that the procedures are appropriate and humane.

For all research conducted on the premises of “Gr. T. Popa” University of Medicine and Pharmacy, including the Centre for Study and Therapy of Pain and the Central Drug Testing Laboratory, the Ethical Research Committee reviews and approves all animal studies. Furthermore, all experiments for teaching purposes performed in the Centre for Study and Therapy of Pain have been video recorded thus eliminating the need for more replicated animal experiments. Students see the videos with the experiments during the classes, tour the facilities of the laboratory and visit the animal housings. However, no animals are experimented upon, strictly for teaching purposes, anymore.

II. **Care and Housing of Laboratory Animals**

When using animals for research, the working conditions with animals, the species involved, the need for their use in the study, source of the animals, number of animals involved in
experiments (we recommend using as few animals as possible), the research protocol (including methods of euthanasia where appropriate, animal situation after the experiment, etc.) will be clearly stated.

The facilities housing laboratory animals should meet current national regulations and guidelines. Laboratory animals must provide humane care and healthful conditions. The researchers and facility personnel are responsible for the conditions under which animals are kept, both within and outside of the context of active experimentation.

III. Sources and Purchasing of Laboratory Animals

Animals needed for the experiments are purchased from specialized and accredited institutions or companies. It is absolutely necessary, in order to obtain accurate and consistent results that can be compared with data obtained in other similar experiments, that identical species (up to any gene changes or knock-outs) are used. Every effort possible has to be made to ensure that the animals are provided with food, water, and ventilation and that they are transported in a non-stressful environment. To ensure the ongoing monitoring of animal-welfare needs, appropriate veterinary care is available and at least a staff member is responsible for the care and welfare of animals.

IV. Personnel

The welfare of the animals is highly dependent on the quality and professional competence of the personnel supervising procedures, as well as those performing procedures or
supervising those taking care of the animals on a daily basis. The staff should be adequately educated and trained before they perform any of the following functions:

1. carrying out procedures on animals;
2. designing procedures and projects;
3. taking care of animals;
4. sacrificing animals;

V. Experimental Procedures

The experiment procedures will detail all aspects of the experiment: choice of species, inclusion and exclusion criteria, methodology used, testing protocol, animal welfare throughout this period, type of experiment - acute or chronic, substances used, desired effect, adverse reactions that may occur, euthanasia method, ethical evaluation and authorization steps.

All procedures can be classified as ‘non-recovery’, ‘mild’, ‘moderate’, or ‘severe’ on a case-by-case basis, using the assignment criteria from European Centre for the Validation of Alternative Methods (ECVAM).

An animal already used in one or more procedures may only be reused in a new procedure provided that the following conditions are met:

- the actual severity of the previous procedures was ‘mild’ or ‘moderate’;
- it is demonstrated that the animal’s general state of health and well-being has been fully restored;
- the next procedure is classified as ‘mild’, ‘moderate’ or ‘non-recovery’; and
- it is in accordance with veterinary consideration, taking into account the lifetime experience of the animal;
The procedure shall be ended when no further observations are necessary, or when the animal experiences pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle. There are exceptions though. In this regard, the International Association for the Study of Pain - IASP’s Research and Ethical Committee, made the following recommendations for animal research in the field of pain:

- the experiments will be conducted in a scientific manner, according to previously established protocols, with clear evidence of the potential benefits;
- in tests of acute or chronic pain, the stimulus will be applied so as to ensure only the minimal pain necessary for the purposes of the experiment;

There are three main reasons for which unnecessary animal suffering occurs. Firstly, when the research protocol is not well justified scientifically or the experiment is badly designed, it could never generate any useful scientific knowledge thus warranting animal suffering too. Second, unnecessary suffering can occur when the extent of animal suffering induced is not strictly required to conduct the experiment (e.g. more animals than necessary are used; less sentient animals could be substituted for more sentient ones, or computer, in-vitro models substitute animals entirely). Third, unnecessary suffering can occur outside the actual research protocol, due to inappropriate animal handling, housing, and feeding practices.

Many models of acute or chronic nociception or pain tests are measured at or near the threshold between necessary pain and suffering, which makes it difficult to comply with these rules. Although it is recommended that all pain research be
Experimental models in rodents

conducted on animals without prior anesthesia, sometimes, in accordance with the ethics of animal experimentation and without influencing the end results, some kind of anesthesia is used. Procedures involving the use of paralytic agents without reduction in pain sensation require particular prudence and ethical concern. The use of muscle relaxants without anesthesia, during surgery, is unacceptable.

Surgical procedures, because of their invasive nature, require a closer supervision and attention to ethical issues. Aseptic techniques must be used on laboratory animals whenever possible. Some general rules on anesthesia and surgery during animal experiments are:

- All surgical procedures and anesthesia should be conducted under the direct supervision of a person who is trained and competent in the use of the procedures.
- Unless there is specific justification for acting otherwise, research animals should be maintained under anesthesia until all surgical procedures are ended.
- Postoperative monitoring and care, which may include the use of analgesics and antibiotics, should be provided to minimize discomfort, prevent infection and promote recovery from the procedure.
- In general, laboratory animals should not be subjected to successive survival surgical procedures, except as required by the nature of the research, the nature of the specific surgery, or for the well being of the animal.
VI. Euthanasia of laboratory animals

The methods and protocols selected for animal research should avoid, as much possible, death as an end-point due to the severe suffering experienced during the period before death.

At the end of the experiment, the animals can have a rapid death, without physical and mental suffering, specific to species and age, according to the AVMA euthanasia Protocol/1993: a painless method to rapidly produce unconsciousness, cardiac and respiratory arrest followed by death. For example, inhalation of a volatile anesthetic (ether or enflurane), which induces unconsciousness within a few seconds is a commonly used method of euthanasia. Other chemical methods include administration of pentobarbital, isoflurane or carbon dioxide. The physical methods used (performed only under anesthesia) include cervical dislocation and decapitation. The absence of vital signs (heartbeat, breathing movements, reflexes) are watched for more than 5 minutes. Prior to the carcass disposal, a secondary means of euthanasia must be performed to assure death. Such methods include cervical dislocation, bilateral thoracotomy, decapitation or exsanguination. Animals should therefore be sacrificed only by a competent person using a method that is appropriate for the species.

A research animal observed to be in a state of severe distress or chronic pain that cannot be alleviated and is not essential to the purposes of the research should be euthanized immediately.

Euthanasia, as a standard procedure as well as the necropsy are performed in special rooms, separated from living animals.
As a conclusion to this chapter, we would like to emphasize that using animals in research is a privilege granted by society to the research community, with the expectation that such use will provide either significant new knowledge or lead to improvement in human and/or animal well-being.

References

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