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# Application of the 3Rs Principles for Animals Used for Experiments at the Beginning of the 21<sup>st</sup> Century

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## Abstract

Passantino A. *Application of the 3Rs Principles for Animals Used for Experiments at the Beginning of the 21<sup>st</sup> Century. ARBS Annu Rev Biomed Sci 2008;10:T27-T32.* In 1986 the Council of Europe adopted Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, which seeks to improve the controls on the use of laboratory animals, to set minimum standards for their housing and care, and addresses in broad terms the training of personnel handling animals and supervising experiments. It also aims at replacing animals with non-animal methods wherever possible, as well as encouraging the development and validation of such replacement methods. Where animals have to be used, the Directive aims to ensure that it is only the minimum number, and that any animal suffering is the minimum necessary to achieve the scientific objective. In view of the important progress made in science at the beginning of the 21st century, of the new techniques now available and of the increased sensitivity regarding animal rights, an updating of the Directive is necessary. In particular, it has become increasingly apparent that the Directive needs to be revised to promote improvements in the welfare of laboratory animals and to further encourage the development of alternative methods. In this review the need to introduce more severe criteria for laboratory experiments on animals, taking ethical aspects into account (specifically the application of the Three Rs principle), is explained. Among the solutions that could improve the control of experimental activity, there could be a cross-check control, made every year, of the number of animals used by the authorized research structures and the number indicated in their scientific output.

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# 1. Introduction

Animal life and welfare are widely considered to be of value and worthy of protection. A “Universal declaration of the Rights of Animals” was promulgated by UNESCO on October 15, 1978<sup>1</sup>. A position, considering animals as public property to be defended, has also appeared.

In recent times, the European Union has taken action on animal welfare and has promulgated some Directives concerning animal protection in experimentation, in breeding, transport and slaughter, etc.

Laboratory animals have value for human usage, but also an intrinsic value in themselves, which should be respected. Animals should be treated as sentient creatures (Passantino & De Vico, 2006; Passantino, 2006, 2007, 2008).

For a long time, animals were defined as agricultural goods (Treaty of Rome, 1957; Anon, 1957).

At last, the European Union Treaty of Amsterdam (Anon, 1997) and of Lisbon (Anon, 2007) defines animals that are kept for commercial purposes as living and sentient<sup>2</sup>, in the sense that they are able to experience pleasure and suffering.

The specific EC Directive 86/609, recognizing that man has a moral obligation to respect all animals and to have due consideration for their capacity for awareness and suffering, sets out common rules incorporating the principles, objectives and main provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Since the Directive dates back more than 20 years, important progress has been made in science and new techniques are now available, such as the use of transgenic animals, xenotransplantation and cloning. Thus, some provisions require specific attention, which the current Directive does not provide, and revision based on more recently available scientific data and current good practices (Binder & Lengauer, 2006).

The revision should address issues such as obligatory authorisation of all experiments, inspections, severity classification, harm-benefit analysis and compulsory ethical review. Also specific problems relating to the improvement of animal welfare should be tackled. Improving animal welfare will often lead to more effective research in terms of its reliability and accuracy, while at the same time it has the potential to reduce the numbers of animals used and to minimise any suffering that may be caused by the scientific protocol.

# 2. Background and Current Legal Situation in Italy

In many countries, the use of animals is subjected to strict legal regulations (Table 1). Individual countries regulate animal experiments in different ways. At the European level, guidelines and legislative regulation of the use of animals for scientific purposes were issued in 1986 by the Council of Europe (Anon, 1986a) and the European Union.

In several European countries, national laws on the protection of animals used for scientific purposes have been issued. For EU Member States the national laws must meet the requirements of the Council Directive 86/609 (Anon, 1986b), which is currently being updated.

Directive 86/609/EEC and its approximation in laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific

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<sup>1</sup> The Universal Declaration of Animals' Rights recognises that animals have rights and establishes that the violation of such rights led and continues to lead man to commit crimes against the natural world; but, above all, it asserts that there cannot be respect among men if at first they do not respect animals. This declaration provides a code of biological ethics for the environment and all the living beings, based on every species' right to live.

<sup>2</sup> The EU Treaty now formally recognises the welfare requirements of animals. The protocol on protection and welfare of animals, annexed to the Treaty of Amsterdam and Lisbon, establishes that “In formulating and implementing the Community's agriculture, transport, internal market and research policies, the Community and the Member States shall pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage.”

purposes aim to ensure that the number of animals used for experimental purposes is reduced to a minimum. When considering the issue of animal testing, the chief objective which must be taken into consideration is the reduction, and wherever and as soon as possible, the elimination of animal suffering.

The elimination of animal suffering is an objective that is common to all parties involved in this issue. This aim corresponds to ethical requirements regarding respect for life, supported by public opinion and it is also included in Directive 86/609/EEC.

Progress has made possible to reduce the number of animals used in experimentation and reduce the level of suffering involved, in line with the “3Rs” concept of replacement, reduction and refinement (Russell & Burch, 1959). This means that efforts ought to be taken to replace the use of live animals by non-animal alternatives<sup>3</sup>, to reduce the number of animals used in experiments to the minimum that is required for obtaining meaningful results and to refine procedures, so that the degree of suffering is minimised.

Scientists have made and continue to make substantial progress in the assessment and improvement of animal welfare and in developing alternative methods to animal use. Also, the scientific community grants that, where the use of animals is unavoidable, every possible effort must be taken to prevent undue suffering and to implement measurements that may improve the animal’s welfare.

In June 2003, the European Commission (Directorate-General Environment) called the first meeting of a Technical Expert Working Group to provide scientific and technical advice on the revision of Directive 86/609, which determines how Member States regulate the use of animals in experiments. The task of the working group was to provide advice on problems with the current Directive and identify potential solutions, providing the arguments in favour of and against each potential solution. The objective of the exercise, as defined by the Commission, was to improve laboratory animal welfare in a way which was applicable throughout the EU, was feasible and practical, given the limitations on resources and the need to limit bureaucracy, and which would permit current, successful regulatory practices to continue ([www.ebra.org](http://www.ebra.org))

In Italy, Legislative Decree (L.D.) 116/92 (Anon, 1992), implementing EEC Directive no. 86/609, currently constitutes the most important reference regarding protection of animals used for experimental or other scientific purposes. This Decree imposes some specific rules for protection of animals used for experimental or other scientific purposes, and considers as offences any experimentation on vertebrate animals which affects their genetic identity, in particular if inflicting unnecessary pain and suffering.

Previous law n. 924 of June 12<sup>th</sup> 1931 and law n. 615 of June 1<sup>st</sup> 1941 forbade, *sic et simpliciter*, experimentation ‘on warm blooded vertebrate animals’ only if it was not aimed at promoting progress of biology and experimental medicine. Since the formulation of L.D. 116/92, legislative protection of animals used for experimental purposes has become wider and more comprehensive. The legislation states that experiments on animals must be carried out in the best conditions. Adequate technologies, structures, environmental conditions, codes of practice and animal maintenance are all essential requirements for practice. Hence, the Decree shows that legislation has developed following the influence of the social and scientific culture, which have become more aware and respectful of animals’ needs and characteristics, including those concerned with behaviour.

These changes are a result of the most modern concepts of ethology and animal welfare. In addition to L.D. 116/92 we can also find this view in Ministerial Circular no. 8 of April 22, 1994, on “protection of animals used for experimental or other scientific purposes”. This Circular states precise rules for all the activities connected with the use of live animals for scientific purposes. The aim of these rules is to reduce the number of experiments involving animals, the number of animals itself and any pain or other poor welfare.

Article 4 of L.D. 116/92 establishes general criteria regarding experiment performance. It also states that all experiments shall be designed directly, or under the direct responsibility of a graduate in

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<sup>3</sup>The concept of “alternatives” has emerged as the primary ethical framework to determine whether the use of experimental animals is required to achieve scientific objectives and whether less stressful or less painful procedures can be used as substitutes for those initially proposed.

Table 1. Regulations on the use of animals in research in European countries.

COUNTRY	LEGISLATION
<b>Austria</b>	1974 - revised in 1989 and 1999
<b>Belgium</b>	1986 - Decret of 14th November 1993, revised 1998 and 2000
<b>Cyprus</b>	1959 -1993, revised in 1995 and 1999
<b>Czech Republic</b>	1992
<b>Denmark</b>	1891 - 4 times revised; last revision in 1993
<b>Estonia</b>	1992 - under revision
<b>Finland</b>	1986 - under revision
<b>France</b>	1987 - Decret n. 848 of 19th October, under revision
<b>Germany</b>	1972 - revised in 1986 and 1998
<b>Hungary</b>	1998
<b>Ireland</b>	Cruelty to Animals Act 1876, revised in 1994 (Statutory Instrument n.17, 1994); under new revision
<b>Italy</b>	Legislative Decree n.116/1992
<b>Luxembourg</b>	1983 - Law of 15th March
<b>Netherlands</b>	1977 - revised in 1997 (Law for Animal Experiments)
<b>Norway</b>	1974 - revised in 1996
<b>Poland</b>	1997 - revised in 1999
<b>Portugal</b>	1992 - revised
<b>Slovak Republic</b>	1995
<b>Slovenia</b>	1985 - revised in 1999
<b>Spain</b>	1988 - Real Decreto of 14th March, n.233
<b>Sweden</b>	1989 - revised in 1998
<b>Switzerland</b>	1978 - revised in 1991 and 1995; currently under revision
<b>Turkey</b>	1996
<b>United Kingdom</b>	Animal (Scientific Procedures) Act 1986, revised in 1993 and 1999

medicine and surgery, in veterinary medicine, in biology, in natural sciences or by anyone having an equivalent degree. Those who carry out experiments or take part in them and those who take care of animals used for experiments must have appropriate education and training and they must be able to handle and take care of laboratory animals.

Furthermore, article 6 states that: "... experiments shall be conducted to avoid distress and unnecessary pain and suffering to the animals", and the veterinary surgeon must supervise "the correct right implementation of experimental procedures"; "decide whether the animal shall be kept alive or killed by a humane method", and "proceed to its elimination when it is impossible to maintain the animal with good enough welfare as specified by article 5".

The Legislator, in this case, did not use the expression "welfare" in a proper way. Experimental activity will sometimes cause poor welfare.

Summing up, the legislator specifies the following aspects:

- the type of experiments in which the use of animals for experimentation is allowed;
- the concept of a specifically responsible person in scientific research;
- the involvement of a veterinary surgeon to ensure the animals' welfare;
- the type of experimentation that requires a specific preventive authorization;
- the environmental requisitioned requests for stalling;
- obligatory registration of the animals used;
- the procedures for communication of experiences to qualified organs;
- the procedures to obtain authorization in quality of user factory.

### 3. Concluding Remarks

One of the principal reasons for Directive 86/609/EEC is to harmonise the implementation of the Three Rs of Replacement, Reduction and Refinement. This would imply that it is important to

define the term “protected animal”, which is to be protected during experimental and other research work.

The concept of the Three Rs aims to ensure that animals are only used when no Replacements are available; that the number of animals used is Reduced to the minimum needed to achieve the scientific objective; and that the experiments are Refined so that only the minimum degree of animal suffering is caused.

Therefore, Reduction and Refinement will help to minimise poor welfare<sup>4</sup> in animals used in experimentation and testing, whilst allowing their use in studies that involve accurate, reproducible, reliable science and are considered justifiable by the competent authorities.

Consequently, research projects should be evaluated in detail for their application of the Three Rs principle, and for the standards of experimental design and research.

In the perspective of identifying barriers to progress, I suggest appropriate solutions to develop, validate and regulate acceptance of alternative approaches, for example:

- mapping of research activities and current strategies;
- cooperation in research to strengthen and enlarge current activities between the partners and other relevant stakeholders;
- development of alternative approaches, including intelligent testing strategies;
- practical mechanisms to improve the validation process using available knowledge;
- practical mechanisms to facilitate the regulatory acceptance process of alternative approaches.

In conclusion, improving animal welfare will often lead to more reliable and accurate research, while at the same time it has the potential to reduce the numbers of animals used and to minimise any suffering that may be caused by the scientific protocol.

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<sup>4</sup> There might be any of a variety of possible causes of poor welfare such as injury, disease and various unfulfilled needs. As a consequence, the animals might feel pain, fear or anxiety or show other coping responses involving brain, behaviour or body physiology. Well, when assessing risks for animal welfare, the Three Rs approach will be used as a general framework.

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