



Guide

for the ethical
evaluation of
experiments using
laboratory animals



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RÉPUBLIQUE FRANÇAISE

**MINISTÈRE
DE L'ENSEIGNEMENT SUPÉRIEUR
ET DE LA RECHERCHE**

**MINISTÈRE
DE L'AGRICULTURE
ET DE LA PÊCHE**

This guide has been sponsored by the Ministry of Higher Education and Research and by the Ministry of Agriculture and Fisheries. It is recommended to be used by researchers needing to use animals in experiments, and committees concerned with the ethics of animal use in experiments

French national committee for consideration of ethics in animal experimentation (Comité national de réflexion éthique sur l'expérimentation animale) considers this guide to be in accordance with the principles adopted in the National Charter on the ethics of using animals in experiments, and is regarded as the reference document for anyone involved in animal experiments.

André-Laurent PARODI

First President

*Comité national de réflexion éthique
sur l'expérimentation animale*

This booklet is dedicated to Chantal Autissier who passed away suddenly in 2008.

Chantal has, since the beginning, actively campaigned for the promotion of ethics committees in France and has inspired Grice for many years. Through her work and enthusiasm, Chantal has been an essential key player in the development of committees.

She is honoured by all her friends and colleagues in Grice.

Grice

The ethics committees' mission is to promote and verify implementation of ethical practices in experiments using animals. In anticipation of, and independent of regulations, these ethical guidelines will reflect the concerns of society and of the scientific community at any given time. They will evolve as a function of the evolution of these concerns, but also as a function of our developing understanding of animals as sentient entities. Therefore, their implementation cannot be based on fixed principles but need to evolve as a function of increasing knowledge and technological progress.

Gircor (Groupe interprofessionnel de réflexion et de communication sur la recherche), pressed by the need expressed by all those concerned and by the authorities involved, asked Grice (Groupe de réflexion interprofessionnel sur les comités d'éthique), the only group in France to stimulate and support the committees, to write a guide to help members of the ethics committees with their evaluation of submitted projects.

This guide, which is not a definite or fixed document, has been acknowledged by the two overseeing Ministries (the Ministry of Higher Education and Research on the one hand and the Ministry of Agriculture and Fisheries on the other hand) as well as by French national committee for consideration of ethics in animal experimentation. This marks advancement in the promotion of ethical evaluation of research projects. In addition, sharing of experience and harmonisation of methods on which it is based contribute to ensure the implementation of principles and good practices shared by all.

Finally, in addition to contributing to the protection of research animals, this guide leads to the improvement of the quality of research, which is a fundamental prerequisite for ethics in experiments using animals

François LACHAPELLE
President of Gircor

GIRCOR

GROUPE INTERPROFESSIONNEL DE RÉFLEXION ET DE COMMUNICATION SUR LA RECHERCHE

- Gircor is an association under the 1901 law, created with the purpose of informing the public of the reasons and conditions in bio-medical research, necessitating experiments involving animals. Gircor is comprised of public and private research establishments. It advocates scientifically valid and ethically acceptable research. Gircor supports Grice and the ethics committees, thus encouraging the development of animal protection within research establishments.

www.gircor.net

GRICE

GRUPE DE RÉFLEXION INTERPROFESSIONNEL SUR LES COMITÉS D'ÉTHIQUE

■ Grice is a Gircor working group. It was founded in France at the beginning of the nineteen nineties and was initiated by Jacques Laurent, a researcher at Roussel-Uclaf. Its objective was to gather private research ethics committees. Grice meets in a plenary session several times a year. In 1993, a charter was drawn up, and in 2000 recommendations were published. Several years ago, the network of ethics committees for public research joined Grice and participates now in its activities.

The National Committee for ethics in animal research was created in 2005 and published the National Charter in 2009. It also aims at raising guidelines for ethics committees.

Besides that, Grice advises the French committees and writes recommendations on practical considerations for the committees' organization and support them in ethical review process, with the will to provide help for the implementation of National Committee guidelines and charter.

http://gircor.net/questions/grice_presentation.php

A Grice working group comprised of the following members has compiled this guide for the ethical evaluation of experiments using animals:

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National Charter on the ethics of experiments on animals

Experiments involving animals are at present indispensable in biological and medical research for scientific, legal and ethical reasons. But this necessity has to take into account that animals are sensitive living beings and, as a consequence, they merit particular consideration.

To this end, Russel and Burch drafted in 1959 the principles of the 3R's, « replace, reduce and refine » which states that, prior to initiating any animal experiment, an assessment should be carried out to check the possibility of replacement or reduction of the use of animals, as well as the means to improve the conditions for the animals.

In 1979, at the European centre of Tufts University in Talloires, the first French charter of ethics of experiments using animals was drafted under the patronage of the Marcel Mérieux foundation, commemorating the centenary of Claude Bernard's death:

« Art 1: Progress of human knowledge, in particular that of biology, human and veterinary medicine is necessary. »

« Art 2: Humans need to use animals in their search for common knowledge, for food, for clothing and for work. It is their duty to respect animals as their co-living beings »

« Art 3: Those involved in biological experiments must be aware that the animal is a sensitive being, has memory and is capable of suffering, without being able to escape the pain. »

The purpose of the ethical evaluation is to ensure that at every stage of a study or a project, the animal is considered as a sensitive living being.

1.1 Presentation of the guide

For several decades, ethics committees for the protection of laboratory animals have been emerging in the world of research. In France, public and private research institutions initiated the establishment of ethics committees and their development.

The mission of these committees is the protection of laboratory animals. Their main means of action are the review of research projects before their initiation. These reviews, known as ethical evaluations, are conducted according to specific well-defined principles. They consist of examining the importance and the scientific quality of the studies, as well as the constraints applied to the animals and the means applied to reduce these constraints when necessary. After the review, a recommendation on the research project is issued.

Considering the complexity and the density of this task, the need for a guide on the ethical evaluation of studies involving animals was expressed by the representatives of the scientific communities and the ministries at the colloquium organized on October 27th, 2005 in Paris by the Gircor, the topic being «The ethics committees for experiments using animals – Situation in France in 2005».

In answer to and on request by Gircor, representatives of all the French ethics committees gathered within the Grice drafted this guide, which was then approved by Gircor.

The guide describes in detail the principles and methods of ethical evaluation of the use of animals for scientific purposes.

The missions, the composition and the workings of the committees are described in other related documents and are not included in this guide.

1.2 The ethics committees

Committees (usually but not exclusively called «ethics committees») which role is to ensure the protection of laboratory animals have been developing for decades throughout the world. In some cases, it is an integral part of the legislation, in other cases it is a supplement to legislation.

The 'Conseil canadien de protection des animaux' (CCPA)/Canadian council on animal care (CCAC) was for example created in 1968 in order to establish the Canadian guidelines on the use of laboratory animals. CCPA/CCAC has drafted a programme compiling a set of recommendations.

The terms of reference requires the creation of Animal Care Committees for all establishments where animals are used for research or for educational or testing purposes. CCPA/CCAC publishes technical documents in French and English on its website.

Similarly, the Institutional animal care and use committees (IACUC) were created in the USA in 1971 by the public institute, the National Institute of Health. Since 1985, they are mandatory in the USA, wherever experiments with animals are performed. These committees have several functions: review of protocols, inspection of premises, semi-annual programme reviews and ensuring personnel training. These committees are the official spokespersons of the overseeing authorities. Note that the IACUC are a supplement to the federal legislation, which does not include legislation on laboratory rats and mice.

In Europe, neither at the level of the Council of Europe (Convention ETS123) nor of the European Union (Directive 86/609), have ethics committees or animal care committees been formally established. The regulations on experiments using animals are based on administrative licensing systems (establishments, studies, researchers) and on recommendations (training, housing, care). The Federation of European Laboratory Animal Science Associations (FELASA) has set up a working group on the ethical evaluation of experiments involving animals. An inquiry was carried out by this working group which, in 2005, resulted in a report stating that 20 European member countries had created ethics committees. In 16 of these countries they are a legal obligation. Harmonisation of the methodology appeared to be necessary. The draft of the revision of directive 86/609 envisages adding to the existing regulations the necessity to use ethical evaluation committees for all experiments on animals or experimental programmes on animals.

In France, the first ethics committees were established in the 1980s. They became common in the Army's research establishments and in the pharmaceutical industry. The latter was supported by Grice's work (publication in 2000 on the activities of the ethics committees in private research, accessible on the Gircor website). In 2001, public research institutions have set up a network of regional ethics committees on experiments using animals (Creeca), which are being reorganized according to the National charter issued in 2008.

All these committees were created to complement the French regulations, which, since 1987 establish the licensing of researchers, the accreditation of establishments and the training of personnel. The goal of creating committees was to add to these regulations a peer-review system for projects and the pooling of best practices for the benefit of the animals and the research. In the context of a national legislation, in which researchers are made fully responsible, it is noteworthy that the creation of ethics committees was spontaneous and voluntary; no official text existed at the time requiring the creation of such committees.

With the aim of harmonizing the principles and the activities of the ethics committees, the CNREEA (Comité national de réflexion éthique sur l'expérimentation animale, National Ethics Committee) was created in 2005. The Committee reports to the CNEA (Commission nationale de l'expérimentation animale, National Commission on animal experimentation) and is commissioned to formulate opinions on the ethical concerns raised by experiments involving animals. It has defined the committees' sphere of activity in regard to laboratories by publishing the National Charter on the deontology and on the ethics of experiments on animals (see Appendix).

1.3 Définitions

For a good understanding of this guide, it is necessary to define certain words. The most frequent synonyms are indicated.

Laboratory animals*:

The expression «laboratory animals» is used in this guide as defined in the article R214-88 of the French rural code. It covers vertebrate animals used for experimental or other scientific purposes.

Welfare:

All the care provided for the animals, aiming at satisfying their physiological and behavioural needs.

Application form:

An application form describes and explains a study or a set of studies. It is drafted by the principal investigator and to be submitted to the ethics committee. It consists of the completed application form and attached appendices, if deemed necessary. The committee's evaluation is based on this application form.

Study:

A study, in the sense of experiments using animals, is a set of procedures performed on animals for a specific purpose: the objective of the study. These procedures may include administration of substances to animals, clinical examinations, data recordings and biological samplings, laboratory examinations of the samples, review of the data by statistical methods.

Also called: experiment, test

Preliminary study:

Study performed to allow the carrying out of full-scale studies. A preliminary study may be necessary for the evaluation, the validation, the adjustment, the adaptation or improvement of a technique or for the refinement of a protocol.

Also called: pre-study, protocol attempt, experimental study, pilot study

Principle Investigator:

Person responsible for a study and for communication with the committee on behalf of this study.

Also called: researcher, study director, applicant

Protocol form:

The protocol form is a template document specific to each committee. The form aims at gathering the information necessary for the committee to make the evaluation review. It is completed by the principal investigator, and is a part of the application form, together with the appendices.

Also called: ethical review protocol, blank application form, animal use protocol, animal use form

Housing:

Housing includes the following areas: the caging system (primary enclosure), the environment (HVAC parameters, illumination, noise), the daily care (feeding, watering system, bedding, cleaning, disinfection), and environmental enrichment.

Also called: husbandry

Standard housing:

The standard housing is the regular housing system of the animals in an accredited unit approved by the competent authority.

Endpoint:

Scientific criterion defined as the point at which a study or part of a study is discontinued in order to limit the experimental animal's pain and/or distress.

Also called: humane endpoint

Procedure*:

A procedure is an intervention or a set of interventions with the aim of achieving a defined objective within the framework of a study. A procedure is often named after its objective (oral administration, urinary sampling). For the purpose of standardisation, a procedure may be described in an internal document (recommendation, operating instruction). This document is sometimes called "the procedure"

Also called: technique, operating instruction

Project*:

Set of studies aiming at the same scientific objective.

Also called: program, research theme

Protocol:

A protocol describes a study. It is drafted by the principal investigator to be used by the staff performing the study.

Also called: study plan, programme of work

* Authors' note:

New references can be found in the Directive 2010/63/EU adopted in September 2010 to revise the Directive 86/609/EEC on the protection of animals used for scientific purposes as regard the definitions of procedure, project and the species considered as laboratory animals. It should also be noted that no definition of study or protocol has been given in the revised Directive.

1.4 General principles of ethical evaluation

The ethical evaluation should enable the assessment of scientific rationale of using live laboratory animals, as well as the choice of the species. The evaluation should allow the committee to ascertain that the principles of animal welfare are respected and that, taking into account the experimental requirements, the conditions of use of the animals are optimized (3R's principles).

Therefore, the ethical evaluation should include an evaluation of:

- the relevance of the animal model with regards to the scientific objective (expected benefit);
- the need to use animals to reach the study objectives (possibility of replacement);
- the likelihood of reaching this objective by the methods proposed;
- the techniques and methods to be used;
- the suffering and distress to the animals, as well as the procedures implemented to minimize these (possibility of refinement/improvement).

Based on these elements, the committee will be able to give an opinion on the study project.

The opinion of the committee should be given before the study begins.

The evaluation may be for a single study or for a set of studies – a project. A standard operating procedure may be reviewed, for example when the pain and/or distress is severe or when it is routinely performed in different studies.

In addition to the preliminary evaluation of a study, the committee may also conduct a retrospective assessment. It consists of examining the development and the results of the study. Retrospective evaluation is particularly recommended for studies which are new to the committee, for long-lasting programmes or for potentially stressful or painful studies. The retrospective evaluation will not be discussed in this guide.

This chapter describes in detail the standard type of ethical evaluation.

The specific aspects of the evaluation of programs, preliminary studies, repetitive studies, outsourced or subcontracted studies and multi-site studies are discussed in chapter 4.

The ethical evaluation elements are presented in the order that corresponds with the logical approach, and most frequently used by the committees in order to reach their goal: compliance with legal requirements, scientific interest and quality, techniques and methodology, suffering and distress, fate of the animals.

2.1 The protocol form

The application form containing the necessary information for the evaluation of a study is submitted to the committee by the principal investigator. This form consists of the correctly completed protocol form and the necessary appendices.

The template of the protocol form should be drafted by the committee and should be comprehensive, precise and concise. The template should be adapted to the research work conducted and to the working method of the committee. Experience shows that this document should be consensual i.e. developed in collaboration with the investigators. Providing instructions is useful in helping the investigators complete the form.

2.2 Legal compliance

Legal compliance monitoring is not part of the ethical evaluation. In France there is no legal requirement to monitor the process of ethical evaluation.

Before submitting it to the ethics committee, the principal investigator should ensure that the scientific project and its implementation conditions comply with the regulatory requirements, in particular, those relative to animal experimentation described in articles R214-87 to R215-10 of the Rural Code.

In order to assess this compliance, the investigator, in the application form, ascertains that the proposed study complies with the regulatory requirements detailed below.

Legality:

The proposed study must be scientifically relevant and cannot be replaced by another experimental method which would not require live animals. The study must be justified with the objective of:

- the diagnosis, prevention or treatment of diseases and other defects in humans, animals or plants;
- the evaluation of the activity, the efficacy and the safety of medicines and of other biological and chemical substances and their composition, including radioelements and materials for therapeutic use in humans and animals;
- the evaluation of physiological parameters in humans and animals;
- quality control of foodstuffs;
- basic or applied research;
- higher education;
- technical education and professional training leading to occupational activities involving experiments with animals or in the care and husbandry of laboratory animals;
- environmental protection.

Persons:

The study must be performed under the direct supervision of the holder of a valid individual license to experiment on live animals. The license must be valid and must cover the requirements of the study. The name of the license holder should be specified on the application form. If the license holder is not the principal investigator submitting the study to the committee, he has to contribute to the drafting of the application form. The authorization to perform surgery should be clearly indicated on the investigator's individual license

Other licenses are necessary, e.g. in the use of genetically modified organisms or of non-domestic species (Certificate of Capacity).

Every animal technician or any person who takes part in procedures on live animals must have attended an accredited training course of FELASA level B (level II in France) or higher.

Every animal caretaker in charge of husbandry and the daily care of the animals must have attended an accredited FELASA level A training course (level III in France).

The facilities: :

The facilities should be approved for the proposed study by the competent local authorities ('agrément d'un établissement d'expérimentation animale délivré par les Services vétérinaires de la Direction départementale de la protection des populations (DDPP)' / accreditation of an establishment for experiments involving animals given by the local governing body for veterinary services). If necessary, the High Biotech Council (national regulatory body in charge of Genetically Altered animals) should give permission to keep transgenic animals and the Prefecture should give permission to house non-domestic animals.

The principal investigator should ascertain that the various licenses are valid for the duration of the study and that they cover the field of study. Unless otherwise specified, (in France,) the accreditation of the study facilities, as well as the individual licenses, and the authorisations for the use of genetically altered organisms are valid for five years. If the duration of the study exceeds the validity of the licenses, the principal investigator has to ensure that the requests for renewal are made in good time.

The animals:

L'étude envisagée doit être conforme à l'agrément préfectoral de l'établissement pour ce qui concerne les espèces animales utilisées et l'origine des animaux.

2.3 The scientific merit and quality

The ethical evaluation of a study includes the evaluation of the scientific merit and the necessity of using animals to achieve the study goals. It also includes the evaluation of the likelihood of achieving this goal by the proposed experiment and its implementation. The more severe the discomfort to the animals, the more demanding the assessment of the scientific justification will be.

One should bear in mind that the ethics committee is not a scientific committee.

The following chapters will address how these two aspects may be reconciled.

2.3.1 Justification of the study

In order to enable the committee to make a recommendation, the justification of the study should be clear. For this reason the principal investigator needs to clearly explain, in the application form, the objectives of the experiment and the anticipated results, even if the importance of the experiment has already been assessed by the institution's scientific authority.

Therefore the investigator needs to explain the context of the study, i.e. the benefits and the relevance within the program, as well as the programme itself.

It is also necessary to demonstrate the relevance of the programme and the study by referring to internal (report of a scientific committee) or external documents (statutory documents, scientific publications, external scientific reviews).

Experimental work is usually original and innovative. Sometimes however, a study that has already been performed needs to be repeated, for example for validation purposes. In this case, the reason should be explained.

The principal investigator should write so that the documents are easily understood by the committee members. Explanations that are too technical or too long should be avoided. Striving for effective communication is crucial. What the public expects from researchers today, in order to grant approval and support is a clear understanding of the expected benefits.

2.3.2 Necessity to use animals

Assessing the necessity for experiments involving animals implies examining possibilities of effectively substituting the experiment by one or several other experimental methods, in order to achieve the intended goals and reaching the conclusion that no other possibility can achieve the prospective results.

On the basis of the information provided on the application form the committee examines this aspect.

It is therefore the principal investigator's duty to demonstrate, with simple biological arguments or with statutory references, that the intended goals cannot be achieved by any other methods than the animal experiment.

It is recommended to attach a scientific publication or the results of a scientific document reference search that supports the arguments to the application as an annex.

2.3.3 Likelihood of achieving the objectives

The assessment of the likelihood of achieving the objectives of the study may appear to be the most delicate part of the ethical evaluation. This evaluation may indeed require scientific expertise which is not always available within the committee.

Several pieces of information should be provided in the application form to substantiate the success of the study:

- Synopsis or chronogram of the study and the experimental design;
- Selection of the animal species;
- Number of animals and statistical analyses.

Synopsis or chronogram of the study and the experimental design:

The synopsis or chronogram of the study uses a diagram to describe the chronology of the various interventions and procedures performed on the animals. It gives a precise idea of the progression and the duration of the study.

It also allows for the documentation of the most stressful stages for the animals. It is a decisive element for the continuation of the evaluation, in particular in the review of the efficiency of the methods applied to reduce the impact of the animals' discomfort.

The experimental design describes the scientific rationale of the study. A study is often based on a comparison between various experimental conditions (compounds, doses, time of analysis). It is advisable to ensure that the experimental design will allow this comparison, notably by laying out in advance the various experimental groups planned for the study.

Selection of the animal species:

The selection of the animal species for the study is justified by the principal investigator in the application form on the basis of available scientific and statutory data. Usually this information will automatically determine the selection. The researcher ensures that, in case of non-domestic species, the choice of species does not threaten biodiversity.

If these criteria allow for a choice between several species, the principal investigator will give precedence to the species that will suffer the least from the discomfort and housing conditions imposed by the protocol.

The principal investigator ascertains that the authorized housing conditions available on the site of the experiment are compatible with carrying out good study (availability of the premises, specific husbandry).

Number of animals and statistical analysis:

A study has to include the appropriate number of animals necessary to reach its scientific objective.

The number of animals used in the study is justified on the application form by the principal investigator. When possible, the number is specified for the entire study as well as for each animal group within the study.

One of the key principles included in the 3R's, which is strictly supervised by the committee, is the effort to limit the number of animals used in the study to the minimum amount necessary and sufficient for the scientific validity of the study. One should keep in mind that a study conducted with a number of animals insufficient to come to a conclusion, is useless.

It is therefore essential that the ethics committee ascertains that the principal investigator has adequately justified the number of animals and that the number does not ensue from the simple replication out of habit nor from previous studies nor from an excessive methodological caution.

The various criteria available to the investigator in order to justify the number of animals are:

- biostatistics;
- regulations and guidelines;
- scientific documentation;
- a preliminary study.

A combination of these different criteria is possible.

2.3.4 Processing the results

The principal investigator describes the methodology used to analyze the biological parameters of the animals during and at the end of the study. This methodology should allow optimal exploitation of the proposed experiment and allow the maximum amount of information to be recorded with the objective of reducing the number of animals used and to correlate the different results.

Access to the technological means necessary to proceed to the analysis of the samples and data obtained during the course of study should be assured. Examples could be access to an electron microscope or electronic data analysis systems.

2.4 Techniques and methodology

2.4.1 General principles

The evaluation of techniques and methodology is carried out in relation to relevant references and up to date knowledge or in relation to normative regulatory requirements (pharmacopoeia). In all cases, any procedure likely to improve the well-being of the animals should be favoured.

A research establishment or institution may wish to establish systems of reference which are inherent to their activities. They will be assessed by the committee.

Any deviation from the references to support the framework of a study should be explicitly reported on the application form by the principal investigator and justified in order to allow the committee to formulate a recommendation.

2.4.2 Housing

Standard housing is defined according to recommendations which supplement the regulations. Adherence to these directives should be guaranteed by the head of each institution. Monitoring the compliance of these directives is ensured by the local governing body for veterinary services.

Any alterations to the standard housing conditions during a study should be documented and be justified in the application form. The most frequent changes applied are isolation, fasting and restriction of mobility.

The committee may recommend compensation (environmental enrichment) or determine a limit of the duration of these specific conditions.

Even in the absence of alterations to the standard housing, the committee may demand improvements of the animals' housing conditions. For example, it may demand a quieter environment for sensitive animals or warmer conditions for weakened animals.

The committee has to know the standard animal housing conditions (cages, aviaries, enclosures, groups, food, and environment) in order to be able to assess the conditions during the studies and to consider the specific arrangements.

2.4.3 Administration of substances and sampling

Most of the studies consist of the animals being exposed to substances or specific environments under precise qualitative and quantitative conditions relative to the intensity (doses), administration site and time (frequency, duration). Likewise, blood sampling is included in many study protocols.

The application form has to specify the administration methods (route, volume, frequency) of administration and sampling for the proposed study.

In the administration of substances or of sampling, it is essential to apply an appropriate technique in a professional manner, enabling the achievement of the anticipated results whilst causing minimum distress to the animals.

Regulations do not specify references concerning administration of substances and sampling. The conditions described in the systems of reference are recommended.

The blood sampling procedure in the retro-orbital sinus of rodents is very frequently used because it allows fast sampling of a quantity of blood sufficient for most tests. Grice would like to stress that this technique, which is applied to a large number of animals, may cause lesions and pain. For this reason, it should be performed under general anaesthetic, which also makes sampling easier. The number of samplings should be restricted.

The physico-chemical properties of administered substances and their vehicle need to be in accordance with the variable biocompatibility criteria for the route of administration: local tolerance, temperature, sterility, osmolality, pH, in order to guarantee the prevention of side effects such as irritation or haemolysis.

Grice recommends A good practice guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes (Technical group of EFPIA / ECVAM, Journal of Applied Toxicology 21, 15-23, 2001) which is a reference used by numerous committees. A summary of the tables shown in this publication is attached in the annex.

2.4.4 Analgesia and anaesthesia

The execution of a study may cause pain or suffering in the animals.

Article R214-91 of the rural code demands that studies which may cause suffering should be conducted under local or general anaesthetic or under analgesia, except if their application is more traumatizing than the study itself or if it is incompatible with the study objectives. The non-use of anaesthetics or analgesics has to be explicitly justified and may require a declaration for the prefecture in accordance with article R214-91 of the rural code.

The principal investigator should therefore provide on the application form, any relevant information regarding the possibility of the occurrence of pain or suffering during the study (see 2.5.1) and on the measures (analgesia or anaesthesia) which will be taken to avoid or reduce them.

He needs to describe and justify the selected analgesic or anaesthetic methods.

A system of reference of the pain levels and the classes of analgesic agents available was drawn up by the Rhone-Alps regional ethics committee for animal experimentation and published by the CNRS (National Centre for Scientific Research) (<http://ethique.ipbs.fr/sdv/doulanimexp.pdf>).

Anyone involved in administering products used for analgesia and anaesthesia, needs to at least be acquainted with the effects of the products on the proposed animal species, the reactions of animals (detection of the stages of anaesthesia, the stages of recovery, signs of pain) and with the actions to be taken in case of early recovery or overdose.

However, the efficiency of analgesia or anaesthesia cannot be based on a prescription alone. It requires on site monitoring. Theoretical training should therefore be complemented with practical experience.

The application form has to include information on these various aspects.

2.4.5 Surgery

Surgery is a procedure that infringes the physical integrity by incision. It is performed under local, regional or general anaesthetics. It requires specific skills that are acquired through basic knowledge and through specialist training for the procedures.

The principal investigator has to specify on the application form the reasons for the need for surgery, the details of the procedures (preparation of animals, anaesthesia, analgesia, asepsis, intervention, recovery and post-operative care), the individuals or teams involved (their training and their licenses) and the equipment or the premises used.

The evaluation by the ethics committee is based on the information provided by the principal investigator, including references to procedures or publications, on the committee's knowledge of the individuals involved and the equipment, on the committee's surgical proficiency (anaesthesia, analgesia, asepsis, surgical technique, monitoring) and if necessary on the opinion of an expert.

The conditions of the committee's assessment have to be proportionate to the severity of the surgical intervention (minor or major).

Minor surgical interventions are used for example for the introduction of a catheter or sensing device in subcutaneous or muscular tissues or for the reimplantation of embryos in pseudopregnant females. They often allow the study to be carried out, without being its objective.

If they are performed on a repetitive basis, these simple interventions may be the object of internal procedures. Once approved by the committee these procedures may be referred to on the application form.

Unless they are mentioned in the procedures, premises and individuals involved should be specified for each study. If it proves impossible to specify the individuals and premises a priori, the committee could accept a reference to a team or a group on the premises.

Major surgical interventions are performed for example for the introduction of intra-abdominal or intra-thoracic devices or for research on surgical aspects. Good management of these interventions demands high level equipment and specialized competence. Their invasive character may cause severe post-operative pains if not reduced by the use of analgesia. If the pain cannot be suppressed, the principal investigator has to verify the necessity to make a declaration to the prefecture in accordance with article R214-91 of the rural code.

Considering these aspects, the committee should pay special attention to major surgical interventions.

The competence and the experience of the individuals involved and the quality of the equipment should be examined in detail.

The committee may recommend a preliminary study (see 4.2). This preliminary study, performed on a limited number of animals, helps to establish the possibility of conducting the study under appropriate scientific and ethical conditions. In particular, it helps to verify that all elements are taken into consideration for appropriate treatment of postoperative pain.

2.4.6 Euthanasia

Euthanasia is the act of terminating the life of an animal using a method involving a minimum of stress and pain.

Euthanasia is regularly conducted at the conclusion of studies, either because it is essential for performing the tests or samplings (which is by far the most frequent case), or because there is no other solution for the animals, in particular when their survival would entail prolonged suffering.

On the application form the principal investigator should specify the moment of the animals' euthanasia, the method of euthanasia, the individuals who are to conduct it, the location of the euthanasia being carried out and the samplings or tests that might be performed before the animals' death (for example during the anaesthetic stage that might precede the euthanasia).

The selection of the method of euthanasia depends primarily on the animal species, the samplings or tests that might have to be conducted, the skills of the individuals conducting the euthanasia and the resources available, especially as regard the number of animals concerned.

Reference documents describe the methods of euthanasia available and also indicate for which species and under which conditions they can be used.

Grice recommends Recommendations for euthanasia of experimental animals: Part 1 and Part 2A (Laboratory Animals 30, 293-316, 1996 and 31, 1-32, 1997) which is a reference work used by numerous committees. These recommendations were prepared for the Directorate-General for the Environment of the European Commission in view of the application of directive 86/609.

An electronic version of these documents is available on

<http://www.lal.org.uk/index.php/education-a-training>

In order to guarantee the quality of the operations, the committee may in certain cases approve a restricted list of usable methods of euthanasia which takes into account the premises and equipment available, commonly performed studies and staff training.

The individuals tasked with carrying out euthanasia must receive training in the selected method. Moreover, the facilities where euthanasia is to be conducted should be equipped for optimal working conditions appropriate to the selected method. Any handling preceding the euthanasia should be performed in such a way that it causes the least possible distress to the animals.

2.5 The constraints

2.5.1 Assessment of discomfort, stress and pain

Studies often impose constraints on the animals. This might include discomfort, for example due to a restriction, even momentarily, of freedom of movement, stress due to handling or pain in the case of certain pathology models, for example arthritis. These constraints can still exist even if the best-known techniques and methods are used.

The assessment of the duration and intensity of the constraints, allows for reflection on ways to reduce these constraints if they seem excessive. This reduction may be achieved in different ways: new techniques, anaesthesia, analgesia, adapted housing. The study protocol is modified according to the improvements selected.

The assessment of the constraints also allows the investigator to comply with the statutory obligation to declare protocols causing pain, in accordance with article R214-91 of the rural code.

The assessment of constraints is written by the principal investigator and presented in a particular paragraph of the application form. It is discussed by the committee.

In order to avoid subjectivity and debates, this assessment should be made with references to published documents.

At present, the different repositories published are not standardised and their use may therefore lead to differing assessments. In this context, it is recommended that the committee selects one and insist that the investigators only use this particular

one. This measure is also justified by the fact that the use of a repository requires a certain routine.

Grice recommends the Classification prospective des expériences sur animaux selon leur degré de gravité (catégories de contrainte) published by the Swiss federal veterinary office and updated in 2004. This comprehensive and clear document is the work of experts in the fields of research, animal protection and administration. This document exists in different languages, including French, thus avoiding divergent interpretations due to translation.

An electronic version is available on

<http://www.bvet.admin.ch/themen/tierschutz/00777/00778/index.html?Lang=fr>

A working group report was also issued in 2009, which conclusions were included in the new Directive 2010/63/EU, as Annex VIII dedicated to severity classification of procedures.

An electronic version of this report is available on

http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf.

2.5.2 Searching for a humane endpoint

In certain fields of research the scientific objective can only be achieved with painful circumstances for the animals. This is the case in certain models for infectious diseases or in cancer research. It also applies to certain regulatory studies.

Scientific and regulatory efforts are being taken to substitute the final phase of such studies by an earlier and, for the animals less painful phase, without impairing the scientific value of the study: this is the humane endpoint.

The principal investigator in charge of such a study needs to consider the selection of a humane endpoint.

The humane endpoint can, for example, be a change of weight or food intake, a specific change in behaviour or appearance, a change in cardiac or respiratory activity. Sometimes it is the blood parameters that need monitoring. The definition of a humane endpoint is often based on several criteria.

Various publications propose humane endpoints for certain types of studies and in literature this is increasingly being discussed. Continuous scientific and regulatory observation by the principal investigator will enable him to keep up-to-date on progress in this area.

Being able to quickly and easily detect the humane endpoint is important, in order to avoid any unnecessary suffering. Euthanasia (of the animal) is often the solution. At times, interrupting the experiment or giving treatment helps to rapidly relieve the animal's suffering and is therefore advisable.

Retrospective evaluations of studies with regards to defining a humane endpoint contain a wealth of information for the principal investigators and the committees (on this topic).

Based on the above information, the principal investigator explains on the application form, his/her solution for the eventual necessity of selecting a humane endpoint for the study he is submitting and the committee will evaluate his solution.

2.5.3 Emergency procedure

It is possible that the general condition of an animal deteriorates rapidly and in an unpredictable way during the course of a study. This is the case, for example, in toxicology or pharmacology studies of new substances.

Because of the unpredictable character of such deterioration, it cannot be included in an application form nor assessed by the ethics committee. The purpose of this chapter is to remind us that an emergency procedure should be in place in every establishment in order to ensure animal protection even in unpredictable circumstances.

A procedure should thus define which measures should be taken to detect severe degradation of the general condition of an animal and to react quickly in order to avoid unnecessary pain whilst safeguarding as many as possible the study objectives.

This emergency procedure includes a list of criteria that permits the detection of severe deterioration in the general condition of each species, in addition to the required information flow needed to quickly reach a decision

and the appropriate action, regardless of the circumstances. The references given in the appendix may help in drawing up this procedure.

The emergency procedure is drafted by the establishment or the investigators and presented to the committee which then makes a recommendation. It is then made available and known to all the individuals who will come into contact with the animals. It applies to all the animals within an establishment.

2.6 The fate of the animals

The principal investigator describes, on the application form the fate of the animals at the end of the study.

For different reasons (see 2.4.6), euthanasia is often carried out. In addition to selecting the euthanasia method, the delay between the final samplings or tests and the euthanasia itself should be decided in advance.

The re-use of animals is sometimes possible. It permits saving animal lives and allows the use of animals with well-known biological characteristics. Animals are frequently re-used in certain fields such as pharmacokinetics or when telemetric methods have been used. However, the re-use of animals should comply with regulations, and should consider the animals' health and the requirements for the scientific validity of the studies.

The regulations concerning the re-use of animals are specified in articles R214-91 and R214-92 of the rural code. This code states that a single animal may not undergo more than one painful manipulation without anaesthesia or analgesia.

The principal investigator should also clarify the re-use policy in order to avoid exposing animals to too frequently repeated tests which could cause a deterioration of their general condition and a decline in or the loss of the studies' scientific value. Therefore, the application form should state the number of times an animal is to be re-used, the frequency and a time limit. In order to establish/define these parameters, the principal investigator takes into account, within the context of his research project, the following elements: constraints imposed on the animals in each study, scientific validity, quality of the housing conditions, follow-ups on the animals' health.

A repository for the re-use of animals does not exist. Certain committees define a totality of severity score which should not be exceeded; others decide case-by-case.

In this context, Grice recommends a case-by-case evaluation that is based, among other things, on the advice from veterinarians, as well as on information from internal references.

The returning of animals to freedom is described by article R214-89 of the rural code. This eventuality may be considered for both wild and domestic animals. In the latter case, adoption is the only option. The return of animals to freedom should be such that the quality of the care they will receive as well as the protection of public health and the environment is guaranteed. The Prefecture of the location where the animals will be set free should authorize it.

Recommendation of the committee

After having examined an application form, the committee issues a recommendation that concludes the evaluation.

3.1 Preparation of the recommendation

In the application form the principle investigator should include all information that will enable the committee to formulate a recommendation on the study project. The review of the application form is performed in executive or plenary session, in accordance with the working procedures of the committee.

After a first reading, the committee may require additional information. This is not part of the recommendation, but of the reviewing process. Likewise, requests from the committee for modifications to the protocol during this preparatory stage do not constitute a recommendation. The committee may meet with the principal investigator in order to discuss certain points.

These exchanges, of which the committee keeps a record, aim to avoid any misunderstanding, to improve if necessary the document submitted by the

principal investigator and, if it is an acceptable project, to give it the maximum chances of acceptance

Only at the end of the preparatory phase does the committee formulate its recommendation.

3.2 Forming/setting-up the recommendation

The committee's project recommendation ensues from the review of its different aspects:

- Legal compliance must be guaranteed by the principal investigator;
- The scientific relevance and value should be adequately established;
- Techniques and methodology should be acceptable;
- The constraints, as well as the fate of the animals, should be documented and acceptable.

The recommendation is attained by a majority vote of the entire committee.

The recommendation may be positive (in favour of implementing the study) or negative (not in favour of implementing the study). There is no conditional recommendation because the conditions were discussed during the preparatory stage of the review.

The recommendation refers unambiguously to the approved project. This is particularly important where successive versions of the study project were presented during the preparatory stage of the review or when the principal investigator introduces modifications after the approval.

The recommendation is well-founded and may include a comment on or a summary of the discussions with the principal investigator.

It may include the expression of minority views.

It can be amended if the principal investigator brings new elements to the attention of the committee.

The recommendation of the committee remains valid for the duration of the proposed project. However, this validity should be limited in time, taking into consideration statutory, scientific and technical developments, as well as the

evolution of the ethical criteria. Consequently, the validity of the recommendation should not exceed 3 years, unless an exception is justified. The duration of validity may be specified in the recommendation. If the study or programme has not finished at the end of this period, a new ethical review should be performed.

The recommendation is conveyed to the principal investigator.

All significant change to the project will change it into a new project, which will require a new recommendation by the committee before it is initiated.

In certain cases, the ethical review raises specific questions that will be treated in this chapter.

4.1 Programme

A programme is a coherent project which aims at achieving a scientific objective and which comprises several studies.

The review of a programme is based on an application form that contains information on all the studies within the program. The committee's recommendation relates to the program.

The review of a programme offers advantages in comparison with that of a study. The scientific relevance and the rationale of a programme are sometimes easier to evaluate than those of a study. Furthermore, in the context of the 3R principle, the review of a complete programme allows a better appreciation of the complementary nature of different methodological approaches (in silico, ex vivo, in vitro and in vivo).

It is up to the principal investigator to decide whether to request a review of the entire programme or a review of each study of the programme separately. The choice will depend on the structure and complexity of the programme or studies. The aim is to find the best possible balance between rationality and quantity of information to be included on the application

form. A programme that is too substantial might be too diverse or too arduous for a review. On the other hand, a study that is too isolated will require complementary information in order to comprehend its relevance.

The duration is also an element that influences the choice. A programme might take many years, whereas the validity of the committee's recommendation should not exceed 3 years. If the programme is continued after the expiry date, it should be reviewed again.

It is often difficult to estimate precisely the number of animals required at the time of the program's project submission. It is the principal investigator's responsibility to define the minimum and maximum values and the decision criteria.

Depending on the complexity or on certain technical aspects of the program, the committee might request, in its recommendation, new reviews at certain stages, or it might demand that information be provided to them during the process of the experiments.

4.2 Preliminary study

In certain cases, preliminary studies are necessary for the achievement of other studies, for example in searching for a humane endpoint, in identifying the variability of a parameter in order to justify the number of animals to be used, in substantiating the correct selection of an animal species, in adjusting the dose range of the product being studied, or in verifying the efficiency of the anaesthesia or analgesia protocol.

Because of the investigative character of a preliminary study, the application form might lack accuracy on certain points. The principal investigator indicates that, for these points, decisions will be made in the course of the study. Depending on the outcome of the observations, he explains how the decisions will be made. This enables the committee to assess if the best conditions are met in order to allow the collection of information needed, while still ensuring the protection of the animals. In its recommendation, the committee may demand to be kept informed of the decisions before they are put into effect.

Since preliminary studies are per definition a prerequisite for the main studies, the validity of the committee's recommendation for these studies should not exceed one year.

4.3 Repetitive study

Often similar studies are conducted in which only the tested product and administration doses differ. This is the case in pharmacological selection (screening) tests. It is also the case in regulatory safety tests (toxicology).

The principal investigator may choose to request the review of a series of similar studies (repetitive or standard study) using a single application form. This choice is based on the similarities of scientific justification, methodology and techniques applied.

The application form for a repetitive study contains all the information required for the review of a classic study, but for one or several items, a range of possibilities and not the precise information is given.

For example, instead of specifying the name of the substance to be tested and its status, the principal investigator describes a group of substances with a specific characteristic. This characteristic may be an activity which justifies a pharmacological study or a developmental stage which may require a toxicology study.

Similarly, in the case of blood sampling, the principal investigator may indicate a maximal and minimal number instead of specifying a fixed number of samplings. The criteria, which will determine the exact number of samplings to be conducted for each study, will be clearly specified.

Therefore, in the case of repetitive studies, the principal investigator should indicate the predicted variations and define the limits. Any modification in the range of these variations, occurring after the committee's recommendation makes the recommendation invalid.

Depending on these elements, the committee may accept the repetitive study or may prefer to review the studies separately, if it considers that the proposed variations are too extensive to be globally approved.

A repetitive study may be repeated during several years. If the study continues further than the end of the maximum period of 3 years or of a shorter period defined by the committee, the study will have to be reviewed again.

4.4 Central service – External sub-contracting – Multi-site studies

A principal investigator subcontracts a study when he decides not to have the study conducted by his own team.

Subcontracting covers different aspects. It applies to a central research service or to an external establishment. There are also cases where different parts of the study are performed in different research centres.

In the case of a central research service, the principal investigator and the head of the central research service jointly draft the application form that contains the usual items described in this guide. They each draft those parts that relate to his/her area of expertise.

In the case of subcontracting to an external establishment, the principal investigator and the representative of the subcontracting establishment concur, in accordance with the establishment's agreements, the selection of the committee to issue a recommendation. Then, exactly as in the previous case, both parties draft the application form.

In the case of a study being conducted on several sites, a single committee is selected to review the protocol, and then the different parties draw up the application form.

The establishments should guarantee that the documents and information needed for drafting the application form (protocol form, instructions, and repositories) are at the disposal of the principal investigators.

An electronic mailbox or letterbox for submitting the application forms to the committee should be at the disposal of the principal investigators. The committee's president organises their distribution to and processing by the committee members.

The review of the application forms is often performed by a sub-committee designated by the president in function of its expertise. The evaluation performed by a sub-committee is communicated to the president.

Requests for additional information or amendments are conveyed in writing to the principal investigators. The committee's operation methods should allow tracing of decisions and interventions. This traceability guarantees the transparency and independence of the committee.

The entire committee makes the final recommendation (see 3.2). It is drafted by its president or a delegate and conveyed to the principal investigator.

The establishments send potential requests for additional distribution to the principal investigators

The establishments define the confidentiality policy of the debates and the recommendations.

The principal investigator ensures information being given back to the committee in accordance with the requirements of the recommendation.

Therefore, in order to achieve its mission of ethical review of studies, a committee needs appropriate human and logistic resources. It is the responsibility of the establishments, who guarantee the proper operation of the committees, to ensure that these resources are made available.

The creation of one committee for several establishments is an opportunity to rally these resources, keeping in mind that several establishments generate more activity than one single one and therefore demand more human resources.

This Guide was developed to help ethics committees with their review of animal experiments. It is the result of the French ethics committees' efforts.

Originating from a need expressed by all participants and regulatory authorities, this guide marks a major step forward in the quality of ethical evaluation of scientific projects. A harmonisation of the methods will contribute to better guarantee the implementation of best practice shared by all.

The protection of animals used in research will benefit from this progress, as will the quality of the scientists' work.

Key documents

- French regulations on animal experimentation
<http://gircor.net/recherche/reglementation.php>
- National Charter on the ethics of using animals in experiments
<http://www.enseignementsup-recherche.gouv.fr/cid28541/la-charte-nationale-portant-sur-l-ethique-de-l-experimentation-animale.html>
- Animal husbandry
<http://conventions.coe.int/Treaty/EN/Treaties/Html/123.htm>
(since 15 July 2007, go to the revised Annex A)
- Functioning of the local ethics committees
<http://www.gircor.net/questions/grice.pdf>
- Functioning of the regional ethics committees
<http://ethique.ipbs.fr/sdv/ethiqueexp.html>
- Regulations for testing medicines (ICH)
<http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html>
- Regulations for testing chemical products (OCDE)
<http://caliban.sourceoecd.org/vl=7788993/cl=21/nw=1/rpsv/cw/vh/osts/oecdjournals/16843681/v1n4/contp1-1.htm>
- Evaluation of constraints
Classification prospective des expériences sur animaux selon leur degré de gravité (catégories de contrainte) (800.116-1.04) - no English version
<http://www.bvet.admin.ch/themen/tierschutz/00777/00778/index.html?lang=fr>

Key documents

Conclusions of the Expert Working Group on severity classification of scientific procedures performed on animals (July 2009)
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf

- Administration of substances and blood sampling
 A good practice guide to the administration of substances and removal of blood including routes and volumes (Technical group of EFPIA/ECVAM. Journal of Applied Toxicology 21, 15-23, 2001)
<http://www3.interscience.wiley.com/cgi-bin/fulltext/76510682/PDFSTART>

Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working group on Refinement (Laboratory Animals 35, 1-41, 2001)
 See the "Refinement and Animal Welfare" section at:
<http://www.lal.org.uk/index.php/education-a-training>

Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement (Laboratory Animals 27, 1-22, 1993 et 28, 178-179, 1994)
 See the "Refinement and Animal Welfare" section at:
<http://www.lal.org.uk/index.php/education-a-training>

- Anaesthesia and analgesia
 Laboratory Animal Anaesthesia. P. Flecknell, Academic Press, 2nd edition (1996).
- Euthanasia methods
 Recommendations for Euthanasia of Experimental Animals, Part 1. Felasa Working Party Report. Laboratory Animals, 30, 293-316 (1996).
<http://la.rsmjournals.com/cgi/reprint/30/4/293>

Key documents

Recommendations for Euthanasia of Experimental Animals, Part 2. Felasa Working Party Report. *Laboratory Animals* 31, 1-32, 1997.

<http://la.rsmjournals.com/cgi/reprint/31/1/1>

AVMA Guidelines on Euthanasia (formerly Report of the AVMA Panel on Euthanasia), June 2007.

<http://www.avma.org/resources/euthanasia.pdf>

Useful documents

- For the ethics committees

Felasa

http://www.felasa.eu/media/uploads/Principles-practice-ethical-review_full%20report%20.pdf

IACUC guidebook

www.grants.nih.gov/grants/olaw/GuideBook.pdf

Canadian council on animal care (CCAC)

<http://www.ccac.ca/>

- Principles of the 3R's

The principles of humane experimental technique. W.M.S. Russell, R.L. Burch. London, Methuen & Co Ltd., 1959.

Balancing Animal Research with Animal Well-being: Establishment of Goals and Harmonization of approaches (James L. Weed, James M. Raber. *ILAR Journal* 46 (2), 118-128, 2005)

Useful documents

- Care and use of laboratory animals

Guide to the care and use of experimental animals (CCAC)

Volume 1:

http://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf

Volume 2 (Authors' note: Volume 2 under revision at the time of publication of this English version of the Gircor Guide):

http://www.ccac.ca/en_/standards/guidelines

Guide for the Care and Use of Laboratory Animals, Eighth Edition (Institute for Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 2010)

http://www.nap.edu/catalog.php?record_id=12910

- Husbandry

Refining rodent husbandry: the mouse. Report of the Rodent Refinement Working Party. *Laboratory Animals* 32, 233-259, 1998.

See the "Refinement and Animal Welfare" section at:

<http://www.lal.org.uk/index.php/education-a-training>

FELASA Working Group Standardization of Enrichment. Working Group Report.

See the "Refinement and Animal Welfare" section at:

<http://www.lal.org.uk/index.php/education-a-training>

- Study design

Festing MFW, Overend P, Gaines Das R, Cortina Borja M, Berdoy M. The design of animal experiments. Reducing the use of animals in research through better experimental design.

Laboratory Animal Handbooks Nr 14, The Royal Society of Medicine Press Ltd., 2002

Useful documents

Fundamental steps in experimental design for animal studies.
de Aguilar-Nascimento JE. Acta Cir Bras. 2005 Jan-Feb;20(1):2-8.
<http://www.scielo.br/pdf/acb/v20n1/23280.pdf>

Guidelines for the design and statistical analysis of experiments using laboratory animals. Festing MF, Altman DG. ILAR J. 2002;43(4):244-58.
http://dels-old.nas.edu/ilar_n/ilarjournal/43_4/v4304festing_b.pdf

- Reduction

Dell RB, Holleran S Ramakrishnan R. Sample size determination. ILAR J. 2002; 43: 207-213.
http://dels-old.nas.edu/ilar_n/ilarjournal/43_4/v4304Dell.pdf

& related erratum:

http://dels-old.nas.edu/ilar_n/ilarjournal/44_3/v44n03erratum.pdf

- Pain levels

<http://ethique.ipbs.fr/sdv/doulanimexp.pdf>

- Humane endpoints

CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing
http://www.ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf

Humane endpoints in animal experiments for biomedical research

See the "Humane Endpoints in Animal Experiments for Biomedical Research" section at:

<http://www.lal.org.uk/index.php/education-a-training>

Useful documents

Humane Endpoints for Animals Used in Biomedical Research and Testing. ILAR Journal 41 (2), 2000.

http://dels-old.nas.edu/ilar_n/ilarjournal/41_2/

OCDE: Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (November 2000)

<http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono%282000%297&doclanguage=en>

The humane endpoint in experiments with animals
CNRS: Le « point limite » en expérimentation animale

<http://ethique.ipbs.fr/sdv/ptlimexpanim.pdf>

Classification rétrospective des expériences sur animaux selon leur degré de gravité (catégories de contrainte) (800.116-1.05)
– no English version

<http://www.bvet.admin.ch/themen/tierschutz/00777/00778/index.html?lang=fr>

Ethical practice in regulated toxicology: Paper on a procedure for deciding humane endpoints. S. Picavet Doctorate thesis in veterinary medicine, Ecole Nationale Veterinaire de Nantes, 2004

Pratique de l'éthique en toxicologie réglementaire : rédaction d'une procédure fixant des points limites. S. Picavet, Thèse de doctorat vétérinaire, Ecole Nationale Vétérinaire de Nantes, 2004.

http://alexandrie.oniris-nantes.fr/GEIDFile/na_04_021.pdf?Archive=192175791035&File=na_04_021_pdf

Useful documents

Guidelines on recognition of pain, distress, and discomfort in experimental animals and a hypothesis for assessment. D.B. Morton, P.H.M. Griffiths. *The Veterinary Record* 116 (16), 431-436, 1985.

- For oncology

UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia (2nd Edition). United Kingdom Co-ordinating Committee on Cancer Research, London 1997.

http://www.ncrdev.org.uk/downloads/MiscDocs/animal_guides_text.pdf

Update in 2010:

Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer* 102, 1555-1577 (2010)

<http://www.nature.com/bjc/journal/v102/n11/pdf/6605642a.pdf>

- For neurosciences

Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (NRC 2003)

<http://www.nap.edu/catalog/10732.html>

- Surveillance tool for animal experimentation

<http://www2.toulouse.inra.fr/versa/>

Recommendations applied regarding the administration of substances and blood samplings.

Ref: A good practice guide to the administration of substances and removal of blood including routes and volumes (Technical group of EFPIA/ECVAM. Journal of Applied Toxicology 21, 15-23, 2001)

The tables below present the quantitative values issued by this publication, in particular regarding recommended volumes. For the qualitative aspects related to the different routes of administration or sampling, as well as to product characteristics, please refer to the original text.

Table 1 : Good practices regarding administration volumes (and maximum tolerated volumes)

Species	Route and Volumes (ml/kg, except for * ml/site)							
	Oral	Sub-cutaneous	Intra-peritoneale	Intra-muscular	Intravenous (bolus)	Intravenous (slow infusion)	Intra-derma	
Mouse	10 (50)	10 (40)	20 (80)	0,05* (0,1)*	5	(25)	0,05*	
Rat	10 (40)	5 (10)	10 (20)	0,1* (0,2)*	5	(20)	0,05*	
Rabbit	10 (15)	1 (2)	5 (20)	0,25 (0,5)	2	(10)	0,1*	
Dog	5 (15)	1 (2)	1 (20)	0,25 (0,5)	2,5	(5)	0,1*	
Macaque	5 (15)	2 (5)	- (10)	0,25 (0,5)	2	(-)	0,1*	
Marmoset	10 (15)	2 (5)	- (20)	0,25 (0,5)	2,5	(10)	0,05*	
Minipig	5 (15)	1 (2)	1 (20)	0,25 (0,5)	2,5	(5)	0,1*	

Table 2 : Recommendations regarding the maximum volume of blood samples, per animal species, per animal species as a function of bodyweight

Table 2a : Circulating blood volumes in laboratory animals

Species	Mean volume (ml/kg)	Interval for mean volumes (ml/kg)	Weight	Blood volume (ml)	7,5 % (ml)	10 % (ml)	15 % (ml)	20 % (ml)
Mouse	72	63-80	25 g	1,8	0,13	0,18	0,27	0,36
Rat	64	58-70	250 g	16	1,2	1,6	2,4	3,2
Rabbit	56	44-70	4 kg	224	17	22	34	45
Dog (Beagle)	85	79-90	10 kg	850	64	85	127	170
Macaque (Rhesus)	56	44-67	5 kg	280	21	28	42	56
Macaque (Cynomolgus)	65	55-75	5 kg	325	24	32	49	65
Marmoset	70	? - 82	350 g	25	1,9	2,5	3,8	5
Minipig	65	61-68	15 kg	975	73	98	146	195

Table 2 b : Limits for volumes of blood samples and recovery time

% circulating blood volumes removed in 24h	Recovery time
7,5 %	1 week
10 %	2 weeks
15 %	4 weeks
20 %	Euthanasia within 24 hours

National Charter on the ethics of animal experimentation*

FOREWORD

Given that animals are sensitive beings, capable of suffering, with cognitive and emotional functions and physiological and behavioural specific needs unique to each species;

given that an alternative method avoiding the use of animals for research, teaching and development of regulatory tests is not always available;

given that in all experimental activities, man should do more than simply apply the animal protection regulations to animals used for experimental and other scientific purposes;

given that, in response to this need, ethical committees for animal experimentation have been created in public and private establishments and that such committees should be more generally established according to common principles;

given that these committees must take into account the principles of the charter as described in article R214-122 of the rural code to formulate their recommendations;

the Comité National de Réflexion Ethique sur l'Expérimentation Animale (French national committee for consideration of ethics in animal experimentation) proposes the following Charter to serve as a reference for researchers and their collaborators as well as institutions and ethical committees.

Article 1 : Respect for the animal

The ethics of animal experimentation is based on the duty that Man has to respect animals as living and sensitive beings.

Article 2 : Individual responsibility

Any use of an animal for experimentation engages the moral responsibility of each person involved.

Article 3 : Responsibility of institutions

The institutions are morally responsible for experiments carried out on animals in their establishments.

Article 4 : Skills

This responsibility involves, at all levels of intervention, an ethical training and regulatory scientific and technical skills proper to the species used and regularly updated.

Specialised skills from experts in physiology, ethology or medicine should be sought whenever necessary for the animals concerned.

Article 5 : General Principles

Careful consideration of a sound scientific, ethical and societal basis justifying the use of the animal must precede any experimental procedure.

The use of methods and techniques aiming at eliminating or reducing to an absolute minimum the suffering of animals must be considered systematically. The development and promotion of such methods must be encouraged.

Optimisation of living conditions, accommodation and care of the animals used must be permanent and continued throughout the animal's life.

The recommendation of an ethical committee must be requested before conducting any experiment on animals.

Article 6 : Ethical procedure

The use of animals for any experimental procedure must be preceded by careful consideration of:

- the usefulness of the planned experiment with respect to studies performed by others;

* This charter has been proposed by the Comité National de Réflexion Ethique sur l'Expérimentation Animale in 2008

* The term 'animal experimentation' is used in accordance with current legislation (articles R 214-87 to 90 of the rural code)

- the pertinence of the chosen methods and the probability of them yielding tangible results;
- the lack of alternative methods to achieve the same goal;
- the adequacy between the animal models planned and the scientific objectives;
- the extent of animal suffering relative to the expected benefit from the results;
- the biological and cognitive characteristics of the species concerned;
- the need to ensure that the choice of species, in the case of nondomesticated animals, does not threaten biodiversity;
- limiting to a minimum the number of animals required;
- the choice of living conditions, accommodation, care and use of animals, such that their physiological and behavioural needs are respected as much as possible.

Article 7 : The role of the ethical committees

Each ethical committee serves to ensure discussion and consideration of issues.

It gives recommendations on the use of animals in research projects submitted to it, referring to the principles stated in this Charter.

These recommendations are justified and can include additional recommendations.

Each ethical committee contributes to the promotion of the ethical principles laid out in this Charter.

Article 8 : Composition of ethical committees

Each ethical committee brings together multidisciplinary skills, to issue competent advices. Civil society (lay members) and veterinary medicine are represented.

Article 9 : Professional conduct of the ethical committees

Any ethical committee must be independent, impartial and must guarantee confidentiality of documents submitted to it.

It must take into account the advice or recommendations of the *Comité National de Réflexion éthique sur l'Expérimentation animale*.

Annexe

Ethical committees for animal experimentation

The ethical committees for animal experimentation are consulting bodies, with the objective of promoting ethical principles and practices in animal experimentation.

In this annexe, the *Comité national de réflexion éthique sur l'expérimentation animale* (the French national committee for consideration of ethics in animal experimentation) proposes to these committees, researchers and institutions on which they are dependent, ways of applying their national Charter on the ethics of animal experimentation (the Charter).

I - ROLE

The fundamental role of an ethical committee, defined by article 7, is to provide recommendations to all researchers intending to undertake experiments on living vertebrate animals, according to current legislation. Article 7 also indicates that ethical committees must promote the principles formulated in the Charter.

All the establishments for animal experimentation must refer to one, and only one, ethical committee. Several establishments may depend on the same committee by associating with each other to create a joint committee.

II - STRUCTURE

The composition and organisation of an ethical committee must ensure its essential **reliability**.

To achieve this, they require:

- **A multidisciplinary representation** allowing expression of the diverse views. For this purpose, an ethical committee must be composed of, at least:
 - a researcher,
 - an individual taking part in the experiments,
 - an individual involved in housing and caring for animals,
 - a veterinary surgeon,
 - an individual external to the establishment(s) for animal experimentation and who demonstrates real interest in animal protection,
- **competence** appropriate in the area of activity of the establishment(s) for animal experimentation that are referring to the committee. If necessary, the committee can call upon the skills of persons external to the establishment,
- the obligation of its members to respect the **strict confidentiality** of discussions and submitted experimental projects,
- **methodical** analyses based on both scientific knowledge and ethical issues,
- the independence and impartiality necessary to justify and freely elaborate at recommendations; this requires that its members are voluntary and that they may not receive any specific remuneration for this function.

III - FUNCTIONING

The institutions to which the establishments for animal experimentation are associated will give the committees the necessary means to fulfil their role.

An ethical committee must respect time delays for responses, compatible with the requirements of the research activity. The operating procedures guaranteeing this rapidity of response are the responsibility of each committee.

a) Ethical assessment

The ethical assessment of projects aims to guarantee that the studies are carried out in the best conditions possible for the animals, on the basis of current knowledge.

The **experimenter** contacts the ethical committee, by submitting a dossier which contains the necessary and sufficient elements for rigorous evaluation of the project.

A project must be comprised of, at least, a scientific aim, an animal model, one or more experimental protocols and for each protocol methods describing how results will be obtained.

For ethical **assessment** of a project, the committee analyses the scientific aim to determine the ethical acceptability of the model choice, the protocol and the methods involved. However, the ethical committees cannot substitute for the scientific committees of a given establishment.

The ethical assessment is based on various aspects of the experiment:

- preparation of the animal,
- the choice and development of the animal model as well as its use,
- the experimental protocol, which must take into account the sensitivity of the animals as well as species-specific constraints and clearly describe the impact of experimental procedures on the physiological and psychological state of the animals,
- pain stages and human end-points are identified in the protocol and measures planned for the prevention, alleviation or even suppression, of the pain are documented on the basis of established references, whenever possible,
- the use of appropriate statistical tools and analytical techniques, which must allow optimisation of the experimental methods used and to generate the maximum number of interpretable results.

The **recommendations** of a committee have, in principle, a maximum validity of three years; the committee should therefore be consulted every three years, even if no operational changes have been made to a continuing project. This new consultation firstly involves a retrospective assessment which must be presented by the experimenter in the new dossier, and secondly checks whether new procedures are available since the previous submission, which could allow the interventions carried out on the animals to be made more ethical, or even replaced by alternative methods.

The **experimenters** and **the establishments for animal experimentation** are responsible for monitoring the practice of these protocols.

b) Delegated ethical assessment

The ethical committee may delegate a part of the ethical assessment for which it is responsible to a local subcommittee specific to each of its establishments for animal experimentation.

This delegation is only permitted in the following cases:

- when only small changes have been made to a protocol, which had already received a favourable opinion from the ethical committee,
- when the planned protocols are standardised, regulated, managed by a code of good professional conduct or when they do not cause pain to the animal (blood sampling, euthanasia using techniques recognised by the ethical committee).

This local subcommittee must have:

- scientific expertise (adequate with the field of given establishment),
- scientific and technical skills concerning rearing, housing and caring for laboratory animals.

The local subcommittee can only act within the framework of a mission letter from the ethical committee, delegating this part of the assessment. The mission letter details the modes of function, notably concerning the fields of intervention and the activity reports that the unit must issue regularly to the ethical committee.

c) Participation in the promotion of ethical principles

The ethical committees participate in the promotion of ethical principles described in the Charter, and in particular of those laid out below:

- the ethical use of animals for experimental purposes involves optimisation of living conditions, housing and care of animals, notably in line with existing codes of good practice or recommendations and using one-off or continuous input from the expertise of animal specialists as necessary. This should be maintained for the duration of the experiment and during animal lifetime,
- any experimentation involving animals must focus on developing methods aimed at reducing, or avoiding, their use and the related constraints.

The committees should share the knowledge and experience gained on the subject as widely as possible, including cases in which the results are not published.

IV - RELATIONSHIP WITH THE *COMITÉ NATIONAL DE RÉFLEXION ÉTHIQUE SUR L'EXPÉRIMENTATION ANIMALE* (the French national committee for consideration of ethics in animal experimentation)

"Any ethical committee for animal experimentation created by the initiative of a public or private organisation and responsible for giving recommendations on the conditions of use of the animals for experimental purposes, or other scientific purposes, must take into account the principles set out in the charter when issuing their recommendations..." Rural code R 214-124.

Each committee should state its commitment to applying the principles of the national Charter to the secretariat of the national Committee* in the form of declared membership signed by its president. This should detail the composition of the committee and the establishments attached to it, and should contain the following informations:

- Name of the committee
- Name of the president and contact details
- The title of the establishment(s) for animal experimentation attached to the committee (accreditation number(s))
- Number of individuals representative of each of the five categories mentioned in chapter II of the current annexe
- Date that the committee was created
- Number of activities delegated to local subcommittees
- Internal rules, where necessary.

These data are strictly confidential and will be kept by the secretariat of the national Committee; they will not be given out under any circumstances.

The ethical committees will inform the *Comité national de réflexion éthique sur l'expérimentation animale*, of any progress they observe that may improve animal wellbeing or reduce, or avoid, the constraints on laboratory animals.

The ethical committees are devoted to take into account the recommendations provided by the national Committee concerning subjects relating to animal experimentation.

* Secrétariat du Comité national de réflexion éthique sur l'expérimentation animale
Ministère de l'Enseignement supérieur et de la Recherche
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Les comités d'éthique pour la protection des animaux de laboratoire se sont développés dans le monde de la recherche depuis plusieurs décennies. La mission de ces comités est la protection des animaux. Leur principal moyen d'action est l'examen des projets de recherche préalablement à leur mise en œuvre. Cet examen est nommé évaluation éthique des études sur animaux.

En France, le monde scientifique et les autorités ont exprimé le besoin d'un guide de l'évaluation éthique. A la demande du Gircor, les représentants de l'ensemble des comités d'éthique français réunis au sein du Grice, l'ont rédigé.

L'évaluation éthique d'une étude par un comité comprend un examen :

- de l'intérêt du modèle animal dans le but poursuivi
- de la nécessité d'utiliser des animaux pour atteindre ce but
- de la possibilité d'atteindre ce but par les moyens envisagés
- des techniques et des méthodes mises en jeu des dommages et des contraintes subis par les animaux et des moyens mis en œuvre pour les diminuer.

L'évaluation se conclut par un avis du comité sur l'étude.

Ce guide décrit en détails ces différents éléments de l'évaluation. Il contient de nombreuses recommandations issues de l'expérience des comités français et des publications récentes. Il contient aussi les références réglementaires et scientifiques indispensables. Il apportera une aide appréciable aux comités et aux chercheurs.

Parti d'un besoin exprimé par l'ensemble des intervenants et par les autorités de tutelle, ce guide marque une avancée dans la qualité de l'évaluation éthique des projets de recherche. L'harmonisation des méthodes apporte une assurance de mise en œuvre de principes et de bonnes pratiques partagés par tous.

La protection des animaux en recherche va en bénéficier, de même que la qualité des travaux de recherche.

Ethics Committees for the protection of vertebrate animals used in scientific procedures have been in existence in the research community for several decennia. The mission of these Ethics Committees is to safeguard the protection of animals used for regulated scientific purposes. The primary task of the Ethics Committee lies in the review of research proposals before the commencement of the research. This process is called Ethical Review of animal procedures.

In France, both the research community and the regulatory authorities have expressed the value of guidelines for ethical review. Upon request of GIRCOR (Groupe Interprofessionnel de Réflexion et de Communication sur la Recherche), representatives of the single national platform of discussion and support of French Ethics Committees in animal research, called GRICE (Groupe de Réflexion Interprofessionnel sur les Comités d’Ethique), have established this guidance.

The ethical review process of an animal procedure embraces a number of aspects and issues and will review:

- The scientific merit of the animal model in the light of the objectives of project
- The necessity to use animals at all in terms of the objectives of the project
- The possibility of attaining these objectives in the manner proposed
- Materials and methods used that cause possible harm and distress of the animal and the possibilities for reducing these.

The ethical review process is concluded by an ethical advise on the procedure.

This guide describes in detail the different elements of the ethical review process. It contains numerous recommendations gathered from the experience of French ethics committees and various recent publications. Valuable regulatory and scientific references are also provided. Both ethics committees and scientists will appreciate the guidance provided.

Originating from a need expressed by participants and regulatory authorities, this guide marks a major step forward in the quality of the ethical evaluation of scientific projects. Harmonizing principles and standards of the evaluation performed will allow to better guarantee implementation of best practice shared by all.

The protection of animals used in scientific procedures will benefit from it, as will the quality of the science produced.