Guidelines to apply for ethical approval of animal experiments

Jan Langbein[1]
Mette Herskin[2]
Frédéric Dehareng[3]
Emer Kennedy[4]
Kenny Rutherford[5]
Antonio Velarde[6]
Véronique Deiss[7]
Isabelle Veissier[7]

[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany
[2]Department of Animal Science, Aarhus University, Tjele, Denmark
[3]Centre Wallon de Recherches agronomiques (CRA-W), Gembloux, Belgium
[4]Teagasc, Animal and Grassland Research and Innovation Centre, Fermoy, Ireland
[5]Animal Behaviour and Welfare, SRUC, Roslin, United Kingdom
[6]Institute for Agrifood Research and Technology (IRTA), Monells, Spain
[7]Université Clermont Auvergne, INRAE, Saint-Genès-Champanelle, France

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Prerequisites

Animal welfare is a value of the European Union (EU) enshrined in Article 13 of the Treaty on the Functioning of the European Union (TFEU) [1]:

"In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, 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."

In order to eliminate disparities between laws and regulations of the Member States regarding the protection of animals used for scientific purposes, the EU passed the Directive 86/609/EEC in 1986. This directive aimed at ensuring that animals used for experimental or other scientific purposes are adequately cared for and no unnecessary pain or suffering is inflicted to them. These rules were refined with the adoption of Directive 2010/63/EU [2] in 2010. Directive 2010/63/EU harmonises animal research legislation across EU Member States and ensures high standards of animal welfare in scientific research. Directive 2010/63/EU was transposed into national law in each Member State by 2013 or 2014. Member States, which enacted more rigorous measures to protect experimental animals prior to the introduction of Directive 2010/63/EU, can maintain them as long as they do not hinder EU-wide scientific cooperation and trade. So national legislations to protect animals used for experimental or scientific purposes include EU standards as defined in Directive 2010/63/EU and possibly additional national standards.

This chapter presents common aspects applying in all EU Member States and in the UK (since Directive 2010/63/EU was transposed into British law before Brexit), and gives the specifications that apply in each research institute of the SmartCow project.

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National animal welfare regulations for using animals in scientific research in EU Member States related to the SmartCow project

Belgium

National animal welfare act:

- Walloon animal welfare act (2018)
  (Code wallon du bien-être animal)

Directive 2010/63/EU implemented into national law:

- Royal Decree on the Protection of Experimental Animals (2013)
  (Arrêté royal relatif à la protection des animaux d'expérience)
- Law on the Protection and Welfare of Animals (1986)
  (Loi relative à la protection et au bien-être des animaux)

Additional animal welfare legislation:

- Royal Decree on the interdiction on certain animal experiences (MB 23.02.2002)
  (Arrêté royal relatif à la protection des animaux d'expérience)
- Law on various provisions relating to animal welfare, Cites, animal health and consumer health protection (MB 31.12.2012)
  (Loi portant des dispositions diverses en matière de bien-être animal)

Other legislation or information:

- –

Competent authority:

- Regional competence

Denmark

National animal welfare act:

- Animal Experiments Act (2013)
  (Bekendtgøres lov om dyreforsøg)

Directive 2010/63/EU implemented into national law:

- Animal testing Act (2015)
  (Bekendtgørelse om dyreforsøg)

Additional animal welfare legislation:

- –

Other legislation or information:

- –

Competent authority:

- Animal Experiment Inspectorate

France

National animal welfare act:

- French Animal Welfare Act (2013)
  (Décret relatif à la protection des animaux utilises à des fins expérimentales)

Directive 2010/63/EU implemented into national law:

- Regulations on the welfare of animals used for experiments or for other scientific purposes
  (Code rural et de la pêche maritime)

Additional animal welfare legislation:

- Non-surgical procedures, surgical procedures, anaesthesia, experiment planner: (Code rural et de la pêche maritime Chapter IV section 6.3 §2 [R214-101-103] and section 6.4 §2 [R214-114-116])

Other legislation or information:

- National Charter for members of ethics committees

Competent authority:

- Ministère de l'enseignement supérieur, de la recherche et de l'innovation (MESRI)

Germany

National animal welfare act:

- Animal Welfare Act (2006)
  (Tierschutzgesetz; TSchG)

Directive 2010/63/EU implemented into national law:

- Regulations on the welfare of animals used for experiment or for other scientific purposes (2013)
  (Tierschutz-Versuchstierverordnung; TierSchVersV)

Additional animal welfare legislation:

- -

Other legislation or information:

- -

Competent authority:

- Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (LALLF)

Ireland

National animal welfare act:

- Animal Health and Welfare Act (2013)

Directive 2010/63/EU implemented into national law:

- SI No 543 of 2012 : European union (protection of animals used for scientific purposes) regulations 2012

Additional animal welfare legislation:

- -

Other legislation or information:

- -

Competent authority:

- The Health Products Regulatory Authority (HPRA)

Netherlands

National animal welfare act:

- Animals Act (2011)
  (Wet Dieren)

Directive 2010/63/EU implemented into national law:

- Animal Experiments Act (1977)
  (Wet op de Dierproeven; WoD)
- Animal Experiments Order
  (Dierproevenbesluit 2014)
- Animal Experiments Decree
  (Dierproevenregeling 2014)

Additional animal welfare legislation:

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Other legislation or information:

- -

Competent authority:

- Central committee animal experiments (CCD) - for licencing projects
- Netherlands Food and Consumer Product Safety Authority (NVWA) - supervision and law enforcement

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Spain

National animal welfare act:

- LAW 5/1995, of June 21, 1995, of protection of animals used for experimentation and other scientific purposes (DOGC No. 2073 - 10/07/1995)
  (LEI 5/1995, de 21 de juny de 1995, de protecció dels animals utilitzats per a experimentació i per a altres finalitats científiques [DOGC núm. 2073 - 10/07/1995])
- DECREE 214/1997, of 30 July, which regulates the use of animals for experimentation and other scientific purposes
  (DECRET 214/1997, de 30 de juliol, pel qual es regula la utilització d'animals per a experimentació i per a altres finalitats científiques)

Directive 2010/63/EU implemented into national law:

- Royal Decree 53/2013, of February 1st, which establishes the basic rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching
  (Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia)

Additional animal welfare legislation:

- Order ECC/566/2015, of March 20th, which establishes the training requirements that must be met by personnel who handle animals used, raised or supplied for experimentation and other scientific purposes, including teaching
  (Orden ECC/566/2015, de 20 de marzo, por la que se establecen los requisitos de capacitación que debe cumplir el personal que maneje animales utilizados, criados o suministrados con fines de experimentación y otros fines científicos, incluyendo la docencia)
- Royal Decree 1386/2018, of November 19, which modifies Royal Decree 53/2013, of February 1, which establishes the basic rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching
  (Real Decreto 1386/2018, de 19 de noviembre, por el que se modifica el Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia)

Other legislation or information:

- -

Competent authority:

- Regional competence, e.g. in Catalonia the competent authority is the Government of Catalonia (Generalitat de Catalunya)

United Kingdom

National animal welfare act:

- Animal welfare Act 2006 (England, Wales)
- Animal Health and Welfare (Scotland) Act 2006
- Welfare of Animals (Northern Ireland) Act 2011

Directive 2010/63/EU implemented into national law:

- The Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (SI 2012/3039)

Additional animal welfare legislation:

- Code of practice for the Housing and Care of Animal Bred, Supplied or Used for Scientific Purposes

Other legislation or information:

- -

Competent authority:

- The Home Office (England, Wales, Scotland)
- Department of Health from Northern Ireland (Northern Ireland)

Directive 2010/63/EU

Directive 2010/63/EU allows the use of experimental animals in the EU only where there is a clear scientific justification, where the expected benefits of the research outweigh the potential risks of animal suffering, and where the scientific objectives cannot be achieved with alternative methods. Only projects that meet these requirements are allowed. The use of experimental animals is limited to specific research purposes, including basic research, applied research on humans and animals, protection of species and the environment, education and training. Directive 2010/63/EU emphasises the need to implement the 3Rs principles of Replacement, Reduction and Refinement and lays down the legal principles:

- replacing experimental animals with non-animal methods where possible,
- reducing to a minimum number of animals used, while at the same time achieving scientifically valid results,
- refining practices to reduce the severity of pain and suffering of experimental animals.

Directive 2010/63/EU classifies the severity of experimental procedures according to pain, suffering, distress or lasting harm. It distinguishes four categories: 'non-recovery', 'mild', 'moderate' or 'severe' (Table 1). The severity of a procedure must be estimated before it is applied on animals (for project authorisation) and monitored when it is applied (for project reporting). Article 16 specifies that animals can be used in several procedures only if these are not severe.

For more explanation on the 3Rs principles, see hapter 'Ethics in experiments on live cattle: a pragmatic approach' [3].

Table 1: Categories for severity of procedures (from Directive 2010/63/EU Annex VIII)

Severity	**Description**
Non-recovery	Procedures performed entirely under general anaesthesia from which the animal shall not recover consciousness.
Mild	Procedures that result in animals experiencing short-term mild pain, suffering or distress, or procedures with no significant impairment of the well-being or general condition of the animals.
Moderate	Procedures that result in animals experiencing short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress or procedures that are likely to cause moderate impairment of the well-being or general condition of the animals.
Severe	Procedures that result in animals experiencing severe pain, suffering or distress, or long- lasting moderate pain, suffering or distress or procedures that are likely to cause severe impairment of the well-being or general condition of the animals.

Animals used in research are to be kept in facilities specially built for this purpose. This applies to breeders, suppliers and users such as universities and research institutes. The facilities must meet a range of general and species-specific requirements for the care and accommodation of animals (listed in Directive 2010/63/EU Annex III). Authorisation by the federal or national licensing and supervisory authorities is required. All institutions that breed, keep or use experimental animals for research purposes shall be regularly monitored by the competent authorities by means of announced or unannounced inspections to ensure compliance with requirements. These institutions should have a designated veterinarian or animal welfare expert (in some countries like UK only a veterinarian can play this role) and an animal-welfare body (AWB). The veterinarian or animal welfare expert advises on animal well-being and treatment. The AWB is an internal committee that provides guidance and oversight on the day-to-day application of the 3Rs principles, monitors the work in progress, and reviews its outcomes [4]. The AWB may help to prepare project proposals. The AWB receives input from the veterinarian or animal welfare expert. All staff must be adequately trained before they are allowed taking care of animals, carrying out procedures on animals, designing such procedures, or killing the animals (defined as functions C, A, B, and D). A common education and training framework was agreed among member states [5]. Specifications can vary between countries (Table 2).

Ethical approval is required if the use of animals in research results in pain, suffering, or permanent damage equivalent to or greater than an injection with a needle. A project proposal is evaluated through a harm-benefit analysis by the relevant national competent authority which then decides on granting or not the authorisation. The competent authority relies on the evaluation of the project by an ethics committee. The procedure to submit a request for approval and the arguments to be developed for that are discussed in the next sections.

Table 2: Competence requirements for staff involved in experiments on animals in EU Member States related to the SmartCow project. Federation of European Laboratory Animal Science Associations (FELASA) courses refer to the four functions defined in Directive 2010/63/EU: A - carrying out procedures on animals; B - designing procedures and projects; C - taking care of animals; D - killing animals (see https://felasa.eu/education-training/course-listings for more information)

Country	Staff role	Professional qualifications & expertise required	Training required
Belgium	Carry out procedures	No specific degree required. Requisite competence must be demonstrated.	FELASA A course + 3 days of continuous training over 6 year periods
	Design of experiment	Master degree ensuring a basic knowledge of medical or biological sciences. Requisite competence must be demonstrated.	FELASA B course + 3 days of continuous training over 6 year periods
Denmark	Carry out procedures	University degree in Veterinary Medicine, Medicine, or Natural Science	Training in Lab Animal Science, e.g. FELASA A course
	Be responsible for the study	University degree in Veterinary Medicine, Medicine, Natural Sciences	Advanced training in Lab Animal Science (e.g. FELASA B course)
France	Take care of animals	No specific degree required. Must be supervised until the requisite competence has been demonstrated.	FELASA C course + 3 days of continuous training over 6 year periods
	Carry out procedures except euthanasia		FELASA A course + 3 days of continuous training over 6 year periods
	Carry out euthanasia		FELASA D course + 3 days of continuous training over 6 year periods
	Design of experiments	5-year academic degree in a scientific area relating to the work performed, **OR** 2-year academic degree and 5 years of professional experience under the responsibility of a person holding a 5-year degree	FELASA B course + 3 days of continuous training over 6 year periods
Germany	Carry out non-surgical procedures	University degree in Veterinary Medicine, Medicine, Natural Sciences, other if activities in training are included	Knowledge and skills according to Annex 1 § 3 of the Regulation on the Implementation of Directive 2010/63/EU into national law
	Carry out surgical procedures, Design of experiments	University degree in Veterinary Medicine, Medicine or further education following a university degree in the natural sciences	

	Head, substitute	University degree in Veterinary Medicine, Medicine, Natural Sciences; 3-year animal experiment experience (alternatively FELASA C course)	Additionally meets requirements according to § 30 of the Regulation on the Implementation of Directive 2010/63/EU into national law
Ireland	Take care of animals	No specific degree required	Training and education as outlined in the Directive
	Carry out procedures (except euthanasia)	No specific degree required. They must be supervised until the requisite competence has been demonstrated.	Scientific animal training course approved by Health Products Regulatory Authority (HPRA)
	Take care of animals	No specific degree required.	Specific training as outlined in the Directive + on-the-job training to ensure individuals understand the role and have sufficient knowledge on the care and use of animals
	Carry out procedures (except euthanasia)	No specific degree required. They must be supervised until the requisite competence has been demonstrated.	HPRA approved scientific animal training course, e.g. LAST Ireland, Charles River Campus, TEARAP
	Carry out euthanasia	No specific degree required. They must be supervised until the requisite competence has been demonstrated.	
	Design of experiment	Academic degree in a scientific discipline relevant to the work being undertaken and species-specific knowledge	
	Manage project	Academic degree in a scientific discipline relevant to the work being undertaken and species-specific knowledge + individual authorisation	
The Netherlands	Carry out procedures	Senior secondary vocational education (mbo) or higher professional education (hbo) in laboratory animal science or equivalent (exemption needed)	Demonstrably competent in the use of laboratory animals
	Design of experiments	Academic Master degree in a relevant field with at least the equivalent of 15 ECTS anatomy and physiology	Training in lab animal science, e.g. FELASA B; species-specific knowledge
	Animal care	Vocational training or professional certificate that includes the specific learning modules	Specific learning modules + On-the-job supervised training + 20 h of continuing training every 8 years

Spain	Euthanasia	according to "Orden ECC/566/2015, de 20 de marzo", **OR** specific training course including the specific learning modules for each role according to "Orden ECC/566/2015, de 20 de marzo" plus a certificate of accomplishment after an on-the-job supervised training	Specific learning modules + On-the-job supervised training + 25 h continuing training every 8 years
	Carry out experimental procedures		Specific learning modules + on-the-job supervised training + 45 h continuing training every 8 years
	Design of projects and procedures	Bachelor, master or PhD in Animal Biology, Human or Veterinary Medicine or another discipline which includes animal biology and physiology + specific training course including the specific learning modules for each role according to "Orden ECC/566/2015, de 20 de marzo"	Specific learning modules + 40 h continuing training every 8 years
United Kingdom	Take care of animals	Personal license holder: General Certificate of Secondary Education (or Standard Grade in Scotland) including a biological science OR equivalent academic, professional or vocational qualifications	Home Office modular training (L, E1, K, PIL A & B, PIL C) + training and assessment of competency in individual procedures
	Carry out euthanasia		
	Carry out procedures (except euthanasia)		
	Design experiments		
	Be responsible for the study	Project license holder	Home Office modular training for Project License holders

Procedure to apply for an ethical approval of an experiment on animals

The competent authorities to whom applications must be sent in each country and/or region of the SmartCow project are listed under Prerequisites. Applications for ethical approval shall at least include the project proposal, a non-technical project summary, and information on the elements set out in Directive 2010/63/EU Annex VI (detailed in the next section) to justify the use of animals, explain how the 3Rs approach has been followed, and describe the competence of staff. Applicants must use forms which are specific to the country where the experiment is performed. The forms are generally available from the website of the competent authority, together with guidance on how to complete them. Each research institute may have internal procedures to check the completeness of an application before it is submitted (e.g. **Wageningen University Animal Welfare Body** at WUR). In UK, AWB and ethics committee are the same committee, called **Animal Welfare and Ethical Review Body** (AWERB), the evaluation is thus internal and the forms to be used by applicants may change from one institution to another.

An ethics committee that assesses an application may approve the application or reject it, or may ask for additional information or for refinement of the procedures. If complements are needed, the applicant is invited to send an amended version that will again be evaluated by the ethics committee. In Germany, Denmark, The Netherlands and France, the competent authority is supposed to send a response to applicants within 40 working days, from the date of receipt of the complete application. Note that the absence of a response does not necessarily mean that the project is approved. In any case, the project cannot start before approval is received.

Arguments to be developed in an application for an ethical approval of an experiment on animals

Annex VI of Directive 2010/63/EU provides a list of points to be addressed in an application:

1. Relevance and justification of the following: (a) use of animals including their origin, estimated numbers, species and life stages; (b) procedures
2. Application of methods to replace, reduce and refine the use of animals in procedures
3. The planned use of anaesthesia, analgesia and other pain relieving methods
4. Reduction, avoidance and alleviation of any form of animal suffering, from birth to death where appropriate
5. Use of humane end-points
6. Experimental or observational strategy and statistical design to minimise animal numbers, pain, suffering, distress and environmental impact where appropriate
7. Reuse of animals and the accumulative effect thereof on the animals
8. The proposed severity classification of procedures
9. Avoidance of unjustified duplication of procedures where appropriate
10. Housing, husbandry and care conditions for the animals
11. Methods of killing
12. Competence of persons involved in the project

In an application, the objective of the experiment must be stated clearly, so that an ethics committee can assess the potential benefits. Applicants should provide evidence that the benefits from the scientific knowledge expected from the experiment outweigh the severity of the interventions on the animals and that duplication has been avoided. They should estimate the potential benefits to individual animals under study, to other animals, to humans (health, welfare, economic benefits), or to the environment.

Applicants need to provide evidence that they followed the 3Rs by:

- justifying the use of animals, taking into account the current state of scientific knowledge (including the fact that the experiment does not replicate a previous experiment) and the absence of alternative methods (e.g. routine measurements, metaanalysis of previous results,…);
- providing the exact number of animals used and justifying that number;
- describing precisely all procedures applied on animals (including killing if performed);

- describing the housing, management and care the animals receive during the experiment. We recommend to describe these also before and after the experiment and to indicate the future of the animals (reuse? killing after a certain period? re-homing?);
- assessing the harms the procedures or the living conditions of animals (housing, management and care) may induce. A wide range of potential harms should be addressed: thirst/hunger/malnutrition, thermal/physical discomfort, disease/functional impairment, behavioural restriction, anxiety/fear/pain/distress. Harms may be due to an experimental procedure per se, to the handling necessary to perform it, or to the housing or management of the animals. Both the nature of conditions applied, their duration, repetition or interaction between them (between several procedures or a specific housing condition and a procedure) should be considered. Procedures resulting in severe harms that are longlasting and cannot be reduced are prohibited, no matter the importance of the expected results (Article 23 of 2010/63/EU);
- in case of procedures inducing pain, specifying if pain relief will be used (and if not, why);
- in case of procedures that may be stressful (inducing anxiety, fear or distress), explaining how stress has been minimised;
- whenever a procedure can be harmful, explaining why the intended harms on the animals are the minimum required to meet the objectives of the research, and defining precise endpoints (conditions under which an animal or treatment group will be removed from an experiment for its own welfare). This can include the monitoring of specific health, behavioural, growth or physiological parameters.

Readers will find examples of how to meet the 3Rs in Chapter ‘ Ethics in experiments on live cattle: a pragmatic approach' of the present book [3].

Evidence that the staff involved in the experiment complies with the legislation in terms of qualification and training shall be provided (see specifications for each country/region of the SmartCow project in Table 2).

Reporting during a project

In the course of the ongoing project, all treatments that happened to the animal must be verifiably documented. Specific information about the condition of the animals in the project must also be verifiably documented. In various countries, forms for documentation can be downloaded from the relevant authority.

Any changes to an ongoing project must be reported. Small deviations may be assessed by the internal AWB of a research organisation. If a modification leads to the use of animals (e.g. exceeding 10%) or the severity of new intervention exceeds the maximum severity of the interventions in the original application, then a new ethical approval shall be requested.

Conclusion and further thoughts

Animal experimentation is highly regulated in the EU. Similar legislative requirements have been set in many non-EU countries. Each EU country designed procedures to check compliance with legislation, which very often includes the need for approval from an ethics committee and for documenting staff competences. When applying for ethical approval, researchers need to describe precisely what will be done during the project and why, while showing that everything is done to minimise animal harms and no alternative scientific method exists. Some people consider that this writing is only 'paper-work'. They will not take benefit of it. This writing does, however, force researchers to think of what is important to do (what experiment is necessary), what could be done to avoid experiments, what are the best practices, and how to minimise harms to animals. These issues should be considered during the planning of a project so that the project is correctly designed. We encourage collective discussions between people designing experiments and staff in charge of applying procedures, of animal care, or even of animal killing. This will not only benefit to animals by minimising their harms but also to science by ensuring that the results can be adequately interpreted thanks to appropriate numbers of animals and minimum suffering.

References

1. European Union. Consolidated version of the treaty on the functioning of the european union. OJEU. 2012;C326:47-390.
2. European Union. Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. OJEU. 2010;L276:33-79.
3. Veissier I, Deiss V, Herskin M, Kennedy E, Rutherford K. Ethics in experiments on live cattle: a pragmatic approach. In: Mesgaran SD, Baumont R, Munksgaard L, Humphries D, Kennedy E, Dijkstra J, et al., editors. Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium. Cologne: PUBLISSO; 2021. DOI: 10.5680/mcpb019
4. Expert Working Group. A working document on Animal Welfare Bodies and National Committees to fulfil the requirements under the Directive. Brussels;2014. Available from: https://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/animal_welfare_bodies/en.p
5. Expert Working Group. A working document on the development of a common education and training framework to fulfil the requirements under the Directive. Brussels;2014. Available from: https://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf

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