Practical manual for the ethical evaluation of animal experiments

General procedure to submit an ethical matrix to the Ethical Committee for Animal Experimentation (ECAE)

The submission of new matrices, prolongations or adjustments needs to be send to ethischecommissiedierproeven@uhasselt.be, at the **latest 1 week before the date of the monthly meeting**. If sent later, evaluation will be delayed and discussed during the following meeting. Applications submitted with an old version of the questionnaire (and not the newest version found on Google Drive), **will not be evaluated until transposed** to the newest version of the questionnaire. Prolongation or adjustments of matrices already approved in the old form will be evaluated and reviewed **only if an intermediate evaluation (or retrospective when the approved matrix expires) using the new form is added to the adjusted older version of the form**. When a matrix expires, the experimental leader is responsible for sending a retrospective analysis **within 3 weeks** to ethischecommissiedierproeven@uhasselt.be. **If no retrospective evaluation is sent, ordering animals will not be possible until the requested retrospective analysis is submitted.** If this 3 week deadline is not met, ordering animals is suspended for the applicant until submission of the evaluation; if the retrospective analysis is again not submitted before the deadline of the next ECAE meeting, ordering animals will be delayed for the entire research group!!!

The experimental leader (for new *in vivo* experiments) will be invited to discuss the matrix during the monthly meeting of the ECAE and will have to clarify questions raised by the members of the ECAE. Comments and suggestions on the submitted matrix, raised by the ECAE members, will be communicated to the experimental leader during the meeting. A revised version (with changes highlighted) will have to be sent back to ethischecommissiedierproeven@uhasselt.be within 1 week after the meeting (if deadlines are not kept, final approval will be postponed).

ECAE members will examine the revised version within 2 weeks after receipt and if approved, a final version of the matrix will be sent back (via e-mail) to the responsible experimental leader.

The official approval letter of the submitted matrix signed by the members of ECAE will be sent by mail to the responsible experiment leader. The ECAE will store the latest version of the questionnaire for at least 3 years after the end of the animal experiment.

0. General structure of the ethical matrix

The form contains 5 parts:

I. **Identification** card of the animal experiment.

II. **Identification** card of the applicant and personnel involved.

III. **Questionnaire** to be filled out by the experiment leader and to be evaluated by the Ethical Committee on Animal Experiments (ECAE). This questionnaire contains 3 parts:

i. *Description of the animal experiment* mentioning procedures, animal species, probable number of animals and also the framework within which the experiment takes place.
ii. *Weighing of the advantages and disadvantages of the experiment*: It is verified whether the animal's pain or suffering (="disadvantages" or "costs") counterbalances the human interests (="advantages" or "benefits").
iii. *Basic conditions of the experiment*: Regardless of the weighing of the costs and benefits, 3 basic conditions have to be fulfilled in order to designate the experiment as ethically justified: (1) the scientific quality of the experiment needs to be guaranteed (2) the principle of the 3 R's (reduction, refinement, replacement) has to be respected to a maximum extent and (3) the safety of all persons involved has to be guaranteed.

IV. **A non-technical summary** which should be understandable to laymen and should not contain any confidential information.

V. **Intermediate or post-experimental (welfare) evaluation** of the animal experiment.

!! The first page of the card is reserved for the Ethical Committee on Animal Experiments (ECAE).

The experiment name and code correspond to the applicant's data. In the box "Remarks – Questions – Suggestions", the ECAE will note questions, comments, suggestions or eventually conclusions. Also, answers given by the applicant related to these questions or remarks, will be inserted by the ECAE on this page.

A revised protocol is only valid if it is signed by at least the following persons: the responsible experiment leader, the president, the secretary and the animal welfare expert, if this last person is different from the secretary.

I. Identification card animal experiment

Animal experiment:
The corresponding box(es) need(s) to be ticked.

In case of *adjustment/change of an ongoing animal experiment*, the date on which the requested change takes effect has to be mentioned. Before ECAE gives advice on the continuing experiment, an evaluation of the previous experiments needs to be performed: **this is an intermediate evaluation (see V.1.).**

In case of *prolongation of an animal experiment* of which the approval expires, the extended date needs to be mentioned. Before ECAE gives advice on the continuing experiment, an evaluation of the previous experiments needs to be performed: **this is an intermediate evaluation (see V.1.)**.

In case of request of a *new experiment within the same project*, also an evaluation of the previous experiments needs to be performed: **this is a post-experimental retrospective (welfare) evaluation (see V.2.). This is to be completed in the document of the approved ethical matrix of the experiment that has ended.**

II. Identification card applicant and personnel

Laboratory (user):
The corresponding box needs to be ticked.
Research group: this can be a subgroup of the laboratory that is accredited by the Federal Public Service (FOD). If such (international) group exists, the address needs to be always mentioned.

Project and or experiment leader(s): note ALL information (name, education, tel. and e-mail) of the personnel involved in the animal experiment (experiment leaders, lab technicians, caretakers).
Remarks: only one project leader is allowed; a responsible experiment leader is to be assigned. The project leader is not necessarily an experiment leader!!

Examples of recognized training programs:

Experiment leader (FELASA C)	**Biotechnician (FELASA B)**	**Animal caretaker (FELASA A)**	**Training program (course)**
X			Laboratory Animal Science (UGent)
X			Art. 9 functionaris (UM, Utrecht, …)
X			UHasselt, module 1 + module 2 KULeuven, module 1 + module 2
X			recognition by the Federal Public Service (FOD)
-	**~**		UHasselt or KUL, module 1
	X		Graduate Schools (KHK Geel; KH Leuven)
	X		Art. 12 functionaris (NL)
	X		recognition by the Federal Public Service (FOD)
		X	BCLAS (biannually)
		X	recognition by the Federal Public Service (FOD)

Person within the using institution which guarantees the proper training and competence of staff: indicate the name(s) of the corresponding person(s).

Animal welfare body involved in this project: The corresponding box needs to be ticked.

Laboratory where the experiments will be (partly) carried out: this has to be filled out if the experiment is (partly) carried out in another laboratory at another institution. The other laboratory (user) has to be accredited for the execution of this type of experiments and for the use of the mentioned animal species. The conduct of an experiment in different laboratories makes that **each ECAE needs to give its approval** for manipulations executed in the laboratory where it is "responsible". The role and participation of each laboratory participating in the experiment has to be described in detail in the protocol.

III. Questionnaire to be filled out by the applicant for evaluation by the ECAE – scientific context and project description

III.1. Description of the animal experiment

Given the fact that an animal experiment always fits in a broader scientific context, separate levels can be distinguished when describing the animal experiment:

- Projects and final goal (question 2)
- Direct purpose (question 3)
- Precise animal experiment (question 4)

Project: a coherent program, possibly composed by different animal experiments, with the objective to answer a global scientific question.
Final goal (project): the global result or the aimed goal of the project as a whole.
Direct purpose: the direct result of an experiment itself.
Actual animal experiment (manipulation): description of all technical actions executed on an animal or on a group of animals which can possibly cause injury, pain or suffering with the objective to answer a specific scientific question.

Question 1

Used animal species and strain/breeding line: an application may contain different species (e.g. mouse and rat). It is important to also mention the strain of the animals.

Age and gender: Do not forget to mention the age and gender (tick the box) of the animals.

Number of animals: distinguish between animals used for breeding, in vitro, or in vivo use (see below); in case an estimate is made, it has to be as accurate as possible (deviation < 20%).

Genetic status: One should distinguish between genetically modified organisms (GMO) and non-GMO animals. Furthermore, the GMOs can be subdivided into two groups, GMO with pathological phenotype and GMO without pathological phenotype. Whenever a new GMO line is created, it is not known in advance whether or not these animals will display a pathological phenotype. Therefore, these animals will be placed in a separate group, named "GMO with an unknown/new phenotype".

Use: Explain for which goals the animals will be used: *in vitro* experiments / *in vivo* experiments / breeding.

Animal supplier: according to the Council's Directive 2010/63/EU of September 22, 2010, on the mutual adjustment of the legal and administrative stipulations of the member countries regarding the protection of animals used for experimental and other scientific purposes, breeding facilities/supply companies must be accredited. Moreover, mice, rats, rabbits, guinea pigs, hamsters (goldhamster, Chinese hamster), quails, Mongolian gerbils, frogs (Xenopus, Rana), zebrafish, dogs, cats and primates have to be specially bred for experimental purposes.

The name (and accreditation number if applicable) of the supplier has to be mentioned. The experiment leader has to check whether the animals have been transported in conformity with the current legislation (standards, accreditation of the transporter). In case of doubt, the laboratory director has to be contacted.

Re-use: Indicate whether you can use animals that have already been used before and explain/motivate your answer.

Question 2

It is important to make clear in which domain the experiment is situated. In case the experiment is part of a similar project, these results can be linked with the results of experiments carried out earlier: this facilitates the insight into the final goal or the global conclusions. Answer the following questions:

- Project and final goal: the aimed goal of the project in a broad coherent scientific context to answer a global scientific question.
- What is the current status of the research topic, by referring to specific literature
- Why is the study necessary? (scientific, ethical, as well as social arguments)

Question 3

Purpose and originality of the animal experiment: describe the direct purpose of the experiment itself. It has to be pointed out in detail how this experiment or the expected results differ (protocol, objective,…) from already performed experiments with the same purpose.

Question 4

This section is the description of all technical actions executed on an animal or on a group of animals which can possibly cause injury, pain or suffering with the objective to answer a specific scientific question within the project.
This section describes all manipulations in chronological order, mentioning the importance, the duration and the level of discomfort of the different actions. Illustrate this description with an overview of the different experimental/treatment groups (with the number of animals included per group) and make a time schedule.
Also to be mentioned: animal welfare provisions, determination of human endpoints. This is the stage of the experiment in which further animal pain or suffering is ended without compromising the scientific value of the experiment (see also question 19).
In case you perform a standard protocol and animal model used by multiple research groups, make sure there is uniformity in the description of the protocol, the scoring system used and the humane endpoints used.
A document specifically describing the manipulations (e.g. SOP) can be enclosed in order to avoid that always the same manipulations or facilities have to be described in the animal experiment protocol (to avoid errors or inconsistency) !

III.2. Evaluation "costs –benefits"

Basic principle: the interest of humans is more important than the interest of animals, but not to every extent. For each animal experiment, the "benefits" (human interests) and "costs" (animal suffering) have to be weighed. Exceptionally, the interests of the laboratory animals are also availed.

III.2.1. Advantages or "benefits": importance of the research

Question 5

The corresponding box must be ticked (just one). Moreover, the questions in italic need to be answered. For this description, a personal or laboratory document can be enclosed.

III.2.2. Disadvantages or "costs": animal suffering, stress and/or discomfort

Question 6

Animals need to be housed according to the standards described in Directive 2010/63/EU (annex III), or annex 3 of the new Royal Decree, on the protection of laboratory animals. It is possible that the standards cannot be respected during the animal experiment. In this case, the reason and the additional animal discomfort (intensity, duration) have to be described. However, each reduction of the physiological and ethological animal needs has to be limited to a strict minimum.

Question 7

Specific efforts are "extra-legal" measures or provisions with regard to housing (cage dimensions, environmental enrichment, biosafety). Additional precautions are important during the use of genetically modified animals suffering from permanent discomfort (pathological phenotype).

Question 8

Level of pain and discomfort (pain score): each experiment has to be classified according to the level of pain, suffering or discomfort the different animal groups experience. This classification also needs to allow the evaluation of the described care or pain control.

Directive 2010/63/EU (annex VIII), and annex 4 of the new Royal Decree, foresees 4 categories of pain/discomfort:

- Non-recovery (P0): procedures performed entirely under terminal anesthesia from which the animal shall not recover or regain consciousness
- Mild (P1) : short-term mild pain, suffering or distress
  no significant impairment of the well-being or general condition of the animals
- Moderate (P2) : short-term moderate pain, suffering or distress
  long-lasting mild pain, suffering or distress
  moderate impairment of the well-being or general condition
- Severe (P3) : severe pain, suffering or distress
  long-lasting moderate pain, suffering or distress
  severe impairment of the well-being or general condition

The category is based on the most severe effects likely to be experienced by an individual animal after applying all appropriate refinement techniques.

If an experimental group is included for in vitro tests only, for which a level of discomfort is irrelevant, tock the corresponding box 'in vitro'.

Additional factors to be taken into account are:

- animal species and genotype;
- maturity, age and gender of the animal;
- cumulative suffering within a procedure;
- training experience of the animal with respect to the procedure;
- if the animal is to be reused, the actual severity of the previous procedures;
- use of anesthesia and analgesia;

- conditions of the experiment (housing conditions, temperature, prevention from expressing natural behavior, access to food and drinking water) in comparison with the etiological needs of the animals;
- human end-points.

Evaluation of the animal welfare during the experiment: a specific document has to be drawn up and stored in the proximity of the animals. It mentions:

- ***a brief summary of the experiment (or the ethical matrix document)****: ref. protocol, manipulations, important observations, expected level of pain or suffering, name of the person responsible for follow-up, coordinates of the experiment leader and person responsible for the animal welfare (in case of emergency);*
- ***a welfare diary*** *with notes on useful clinical observations (body temperature, breathing frequency, food and drinking water consumption, excretions,…) and ethological findings (abnormal behavior, immobility,…), and all interventions in case of stress, suffering or discomfort*

For the evaluation, the foreseen classification (pain score) can be a useful element.
This "welfare diary" can be prepared according to the following model:

Welfare diary Cage n°: ……

Animal experiment (ref. protocol) : …….
Manipulations (chronology, important observations, expected level of pain/discomfort) :……
……
……
……
Person responsible for the daily evaluation (name, tel.):……..
Person responsible for animal welfare (in case of emergency):…….
……

Date	Observations	Actions taken	Signature

Question 9

It is important to describe the used anesthesia and analgesia in detail (products, concentrations, dosing, administration route, duration, frequency,…) and specify which references were consulted to decide on their use.

Question 10

Euthanasia: the guidelines described in Directive 2010/63/EU (annex IV) or annex 6 of the new Royal Decree, have to be followed. ***The use of any other method has to be scientifically founded or a bibliographical reference is to be given! E.g. in case decapitation is used, this needs to be motivated.***

Question 11

Animal's future after the experiment: in most of the cases, animals are euthanized after the experiment. But in other cases, animals are kept alive and could be reused. One animal cannot

be used twice for a painful or stressful experiment. In general, animals used in a P1 or P2 experiment can only be reused in a P0, P1 or P2 experiment, provided that the general health of the animal has been fully restored and only after veterinary advice. Animals used in a P3 experiment cannot be reused.

III.3. General conditions

III.3.1. Quality of the research

Question 12

The scientific quality of the experiment has to be guaranteed. Every useful reference regarding the source of the used animal model has to be mentioned.

III.3.2. Implementation of the 3 R's

The principle of the 3 R's must always be applied. Equivalent procedures or alternatives by which animals can be replaced by "in vitro models" or by which less animals are needed, have to be used. When setting up the animal model and executing the manipulation, suffering or discomfort has to be maximally reduced.

REPLACEMENT:

Question 13

Research: Several databases need to be consulted for existing alternative *in vitro* methods, such as:

- ECVAM or FRAME
- Invittox
- SIS (http://ihcp.jrc.ec.europa.eu/)
- Go3Rs (searches pub med) (http://www.gopubmed.org/web/go3r/)

Question 14

Regulatory tests:

- if an alternative *in vitro* test was validated by ECVAM (European Centre for the Validation of Alternative Methods) and if the method is recognized by the competent authorities, the execution of an animal experiment is prohibited.
- Every experiment leader is obligated to consult the available lists of validated alternatives (OECD, Organization for Economical Collaboration and Development: http://www.oecd.org/; ECVAM).
- Recent list of alternatives validated by EURL ECVAM (http://ecvam-dbalm.jrc.ec.europa.eu)
- Recent list of alternatives validated by European Pharmacopoeia

Education: the use of animals in this respect has to be justified. It has to be demonstrated that alternative methods (video, …) cannot be used.

- Norina (http://www.oslovet.norecaps.np)
- NCA (http://www.nkca.nl)
- Interniche (http://www.interniche.org)

Question 15

The corresponding box needs to be ticked. If yes, explain which alternatives.

REDUCTION:

Question 16

This does not apply to experiments with a regulatory character. In other cases, explanation is necessary when experiments are repeated under the same conditions (product/molecule, protocol, animal species, …). If this aspect has been mentioned under IV.1. « description animal experiment », refer to it.

Question 17

The exchange of animals between laboratories is only allowed when the laboratories dispose of all necessary recognitions as animal supplier (recognition as "LA2").

Question 18

The research has to be statistically founded where possible. See for example: Principles of Laboratory Animal Science, Van Zutphen et al. (2001), chapter 12.
Contact CENSTAT through Kim Pannemans in case you need help with the power calculation.

REFINEMENT:

Question 19

This question is an addition to question 9 in which specific welfare measures are specified (individual follow up or in group, before and/or during the experiment, frequency of follow up).

Question 20

As stipulated before, the 'human endpoint' is the stage where manipulations are stopped because further animal suffering or discomfort is not useful for the experiment and therefore can no longer be justified. Hence the scientific value of the experiment will not be harmed. **The endpoints always have to be determined before the start of the experiment. Absence of endpoints has to be founded.**

Question 21

By the law of 14th August 1986 on the animal protection and welfare, animal experiments must always use the least sensitive species (neurophysiological parameters). If not possible, the choice of the animal species always has to be founded and justified.

Question 22

All relevant remarks on the use of alternative methods that have not been used before can be communicated to the Ethical Committee here.

III.3.3. Safety researchers and environment

During the experiment or project, the safety of all parties concerned has to be guaranteed.

Question 23

When using dangerous products or organisms, the responsible person within the institute has to be contacted. The links mentioned in question 24 can be useful.

Question 24

Regulation concerning the use or dangerous or genetically modified organisms can be found on the following websites:

- Biosafety: http://www.biosafety.be/CU/NL/CUMenuNL.html
  http://www.hc-sc.gc.ca/pphb-dgspsp/publicat/lbg-ldmbl-96/index.html
  http://www.vib.be/InfoEdu/EN/Biotechnology+and+safety/Regulation/

- Regional legislation: Flanders: http://www.mina.be/amv.html
  the Walloon provinces: http://wallex.wallonie.be/
  Brussels metropolitan region: http://www.ibgebim.be/ or
  http://www.cass.be/cgi_loi/legislation.pl

More information can be found at
http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm

IV. Non-technical summary of the requested animal experiment

Under the new legislation (European Directive 2010/63/EU), each project submitted for evaluation by the ECAE, must be accompanied by a non-confidential non-technical document (Non-Technical Summary, "NTS") which is prepared by the responsible researcher, must be written in Dutch and must be understandable to outsiders (*i.e.* written in laymen's terms). This document contains the following information:

a) Information on the objectives/goal(s) of the project, including the predicted "costs-benefits", the number of animals and the animal species to be used.
b) Proof that the requirement of replacement, reduction and refinement is met.

This information is sent to the Federal Public Service (FOD) annually by the ECAE.

V. Intermediate or post-experimental retrospective (welfare) evaluation of the animal experiment

Intermediate or post-experimental (retrospective) evaluation must be filled in when an adjustment/change or prolongation is requested by the applicant (intermediate evaluation) or when part of the animal experiment (or the complete experiment) is finished (retrospective post-experimental evaluation). A postexperimental/retrospective analysis is requested by law (2010/63/EU directive) and is therefore **mandatory**.
This section compares the evaluation before the start of the animal experiment (planned number of animals to be used, pain level) with the one during or after the experiment. **Adjustments, prolongation or new matrices for new experiments will not be accepted by the ECAE if intermediate or requested post-evaluations are not provided.**