LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture, de la Viticulture
et du Développement rural

Administration des services vétérinaires

Regulatory Guidelines

Application for the Amendment to a Project authorisation of animal experimentation under the Grand-Ducal Regulation of 11 January 2013 for the protection of animals used for scientific purposes

Legal basis:

-Règlement grand-ducal du 11 janvier 2013 relatif à la protection des animaux utilisés à des fins scientifiques

-Loi du 27 juin 2018 sur la protection des animaux

CONTENT:

1. SCOPE

This guidance is intended to assist applicants in completing the form 'Form_Am_Pr_Au'. It is therefore strongly recommended to read this guideline in conjunction with the respective form, which can be found on the website
https://agriculture.public.lu/de/tierhaltung/labosdeieren.html

2. INTRODUCTION

A project must be carried out in accordance with the terms and conditions of the relevant project authorisation. Therefore, and according to article 39 and article 43 of the Grand-Ducal Regulation of the 11 January 2013 for the protection of animals used for scientific, any changes in the project must be subject to a modification of the initial project authorization. In this situation, an application for amendment must be submitted and approved in order to proceed with a substantial change to a project involving the use of animals.

The following cases requires an amendment of project authorisation:
a) a change in the project, which would result in a negative impact on animal welfare (article 43);
b) any changes to the project authorisation regarding article 39 paragraph 2 of the Grand-Ducal Regulation of the 11 January 2013.

In the form, the different cases are specified in the Section C:

- Section C1 -Amendment to project leader,
- Section C2- Amendment to or addition of deputy project leader(s),
- Section C3- Addition of new individuals who will be performing procedures,
- Section C4 - Amendments to existing experiment,
- Section C5 - Addition of new experiment(s),
- Section C6 - Amendments to Species/Strains,
- Section C7 -Increase in the total animal numbers.

A change to an experiment that would potentially result in an increase in the level of pain, distress or suffering (whether alleviated by treatment or not) or the addition of a new experiment to an existing project authorisation will require an application for an amendment of the project authorisation or a new project authorisation. On the other hand, an amendment to a project authorisation is not required for changes that improve the welfare of the animals involved, e.g. reducing the dose of a test substance being administered or improving the animal's environment.

It is important to note that if there is a significant change to the objectives or scope of the project, or in some circumstances the harm-benefit analysis as a result of the proposed changes, a new project authorisation must be required.

Whether or not the planned changes require a new project authorisation instead of a project amendment is decided on a case-by-case basis. Applicants who are unsure whether or not the planned changes require a new project authorisation rather than an amended project authorisation are advised to contact the Administration of Veterinary Services.

An application for an amendment to a project authorisation has to be made to the Ministry of Agriculture, Viticulture and Rural Development. It can be submitted by the user or by the user or the project leader on behalf of the user.

Please note that an application for a project amendment or renewal must be made whether or not the project amendment in question has been approved by the user's ethics committee. The Ministry of Agriculture, Viticulture and Rural Development makes every effort to complete the evaluation of project amendment applications within 40 working days. This does not include any time taken by the applicant to respond to queries sent by the Ministry of Agriculture, Viticulture and Rural Development. In addition, the Ministry of Agriculture, Viticulture and Rural Development reserves the right to extend the time taken to make a decision regarding an application for project amendments to 55 working days, when justified by the complexity or the multi-disciplinary nature of the project concerned. However, the Ministry of Agriculture, Viticulture and Rural Development expects this to be a rare occurrence.

3. HOW TO APPLY FOR AN AMENDMENT OF A PROJECT AUTHORISATION

The form can be found on the website https://agriculture.public.lu/de/tierhaltung/labosdeieren.html . Please download the form, fill it out (provide the details of the project, the name of the project leader, select the purpose of the application 'amendment' and complete the relevant section(s) of the form) and send it back via mail to xxx.

4. HOW TO PROVIDE DETAILS ON PROPOSED AMENDMENTS

This refers to the Section C of the Form

C1: CHANGE OF PROJECT LEADER

If the purpose of the application is to change the project leader, enter details of the proposed new project leader. The proposed new project leader should sign the declaration and undertaking in Section E of the form. The proposed project leader must hold a certificate of education and training for the function B designing procedures and projects.

C2: CHANGE OR ADDITION OF DEPUTY PROJECT LEADER(S)

If the application is to amend an existing deputy project leader(s) or add a new /additional deputy project leader, this should be outlined clearly including the full name of the existing

deputy project leader(s) to be replaced. The proposed project deputy leader must hold a certificate of education and training for the function B designing procedures and projects.

Multiple deputy project leaders can be added by copying and pasting the table in Section C2 as many times as required.

C3. ADDITION OF NEW INDIVIDUALS WHO WILL BE PERFORMING PROCEDURES

Details of all new personnel planning to perform procedures as part of the approved project authorisation must be provided. All personnel must hold a certificate of education and training for the function functions: (a) carrying out procedures on animals; (c) taking care of animals; and/or (d) killing animals.

C4: AMENDMENTS TO EXISTING EXPERIMENT(S)

The suggested experiment amendment(s) should be as detailed as possible. The experiment to be amended must be listed using the procedure number (applicable for all projects submitted under the new form published 2020) and the experiment title. Please indicate clearly, how each amendment proposed differs from the approved experiment(s) and the justification/relevance of the amendment to the experiment.

It may become evident during the evaluation of a project amendment application that the proposed amendment(s) to existing experiments impact(s) negatively on the original harm-benefit analysis. A new project authorisation may be required in these situations, however this will be decided on a case-by-case basis and applicants will be notified by the Ministry of Agriculture, Viticulture and Rural Development as soon as possible.

For multiple procedure amendments, the table in Section C4 can be copied and pasted as many times as necessary.

If, during the course of an authorised project, the severity classification of a procedure is found to be higher than that originally proposed and authorised, this should be reported to the animal welfare body immediately and recorded as a project deviation. If the project is to be progressed with the increased severity classification, an application for a project amendment must be submitted to the Ministry of Agriculture, Viticulture and Rural Development without delay.

C5: ADDITION OF NEW EXPERIMENT(S)

Details of the proposed new experiment(s) must be provided, including a justification as to why the new procedures are necessary.

It may become evident during the evaluation of a project amendment application that the new experiment(s) impact(s) negatively on the original harm-benefit analysis and a new project authorisation may be required in these situations; however, this will be decided on a case-by case basis.

For multiple new experiment(s), the table in Section C5 can be copied and pasted as many times as necessary.

New experiment number:	Name of new experiment:
Select a procedure number beginning with 1	Name each experiment accurately. For example; - 'IV injection of therapeutic substance' (class of substance/ should be named where possible), - 'surgical implantation of tumour cells' (location of implantation should be named), - 'induction of EAE by SC injections of inflammatory adjuvant' (adjuvant used should be named where possible).
Description/details of experiment	

Describe the treatment(s), intervention(s) and manipulation(s) done in the respective experiment. List chronological steps starting with the first preparative step and ending with the death of the animal or the last procedure in the experiment.
If possible, please include a graphical illustration and protocol schedule.

For Example: Title: Skin carcinogenesis

1. (Optional and at any stage) Blood samples may be taken from a superficial vessel.

2. (Optional and at any stage) Skin biopsy up to 3 mm diameter may be taken (Anaesthesia applied). No site will be biopsied more than once with a minimum of two weeks between biopsies; no animal will be biopsied more than 4 times during its life.

3. (At any stage) Immunotherapeutic substances will be administered alone or in combination, continuously or intermittently by one or more of the following routes:
a) in diet or drinking water
b) subcutaneous
c) intraperitoneal
d) implantation of a slow release pellet subcutaneously on one occasion (Anaesthesia applied)
e) topical application

4. Tumour development will be induced by either:
a) topical application of a substance; or
b) UVB exposure

5. (Optional) Some of the animals will be used in non-invasive imaging studies (e.g. NMR, CT) (Anaesthesia applied).
- Animals may receive a single injection of contrast agent prior to or during image acquisition by the intra-peritoneal or intravenous route (Anaesthesia applied).
- Mice will be exposed to imaging sessions typically no more than two times per week (Anaesthesia applied) and for no more than 5 weeks (maximum 10 imaging sessions).

6. Terminal studies and killing:
- Killing by a Schedule 1 method
- Or exsanguination (Anaesthesia applied) followed by killing by a Schedule 1 method
- Or perfusion fixation (Anaesthesia)

Justification/relevance of experiment. Justify why it is necessary to add this new experience and explain how this experiment will contribute to the benefits of the project.

For each experiment to be performed as part of the project, provide details on the **relevance** of each experiment to the overall project objective. Where substances are being administered, both the route of administration and the substance being administered should be justified. Where an animal model of disease is being used, justification for this model over other available models should be included.

Please give information why it is necessary to add this new experiment and explain the **scientific benefit**.
When describing the potential benefits, the following considerations should be incorporated in the details provided:
- a description of the potential benefits, ensuring that they are realistic
- the potential advances in scientific knowledge which could be obtained, and the value of this knowledge
- why these potential benefits are important
- who will benefit.

The ultimate benefits of basic/fundamental research to humans, animals or the environment may not be fully known at the time of applying for a project authorisation; however, the following should be clearly outlined:
- the hypothesis and the supporting evidence for the hypothesis
- previous work in the area and the specific knowledge gap(s) that will be filled by the proposed project
- the expected impact and strength of the research and its expected contribution to the field.

Details of the planned **dissemination of results** (e.g. publication plan) should be provided.

Species	Provide information on the species on which the procedure is to be performed.
Life stage or age	Provide the life cycle stage or age of the animal. Examples of life stages include embryo, larval, neonate, adolescent or adult.
Number of animals to be used	The number of animals proposed to undergo the procedure.
Will this new procedure increase total number of animals required for this project?	Give information if the new procedure will increase the total number of animals required for this project. If the total number will not change, please explain why not.

Frequency of procedure (how many times will the procedure be performed?)	Provide information on the maximum total number of times each animal will undergo the procedure as part of the project. Examples include; - if an animal is undergoing a procedure weekly, information must be provided on how many weeks in total this procedure will be conducted and the maximum number of times an animal will undergo the procedure. - if a procedure is to take place weekly over a six-week period, the entry should state 'weekly for a total of six weeks; a total of six times per animal', rather than simply 'weekly'. If within the project, different groups of animals are scheduled to undergo a given procedure at different frequencies, please give the range, and worst case scenario (i.e. highest frequency), at which any animal will experience the procedure. Please include the time which will elapse between each frequency (i.e. '4 times over a total of 2 weeks', rather than '4 times').
Duration of procedure (how long will the procedure take/how long will the animal be affected for?)	Provide information on the length of time that the procedure will take per animal. If the procedure is a surgery, provide the duration of the surgery (e.g. 'two hours') and the duration of any intended impacts on the animal (e.g. 'four weeks of neurological deficits'). If the procedure is a single injection which induces a disease process, the duration should not be the length of time it takes to administer the injection, but rather the length of time the animal will experience suffering due to the disease induction (e.g. the injection of an arthritis-inducing agent may have a duration of 8 weeks).

Proposed severity classification of the procedure	☐ Non-recovery ☐ Moderate ☐ Mild ☐ Severe Select a severity from the list. Procedures are classified as 'non-recovery', 'mild', 'moderate 'or 'severe 'based on the criteria set out in Article 15 and Annex VIII to Directive 2010/63/EU. The European Commission guidance documents on severity assessment and the 'Illustrative examples for the process of severity classification 'are also useful references in prospective severity classification.
List all the potential expected adverse effects of the procedure. Include the estimated % of animals that may experience each effect listed.	
Describe in detail any potential effect(s) of the procedure that will impact negatively on animal welfare, and the percentage likelihood that each adverse effect(s) will occur. Where substances are being administered, both the adverse effects of the route of administration and the adverse effects of the substances being administered should be included.	
If there is an expected attrition rate, give the estimated % of animals and describe the potential reasons (e.g. reaching humane endpoints, anaesthetic deaths, failure of animal model, other).	
Estimate the percentage of animals that may not reach the pre-determined end-point of the experiment and outline the reasons (e.g. being culled early due to reaching humane endpoints, anaesthetic deaths, failure of animal model, other).	
Relating directly to the adverse effects, list all procedure-specific humane endpoints and give detail about the animal welfare monitoring arrangements.	
All procedures must have clearly defined procedure-specific humane endpoints, directly related to the adverse effects, describing the criteria which will determine when animals will be removed from a study for welfare reasons (e.g. weight loss of 20%, ulceration of tumour, etc.). Provide details about how the welfare of the animals will be monitored and scored throughout the project, including details on the use of score sheets. It is important to note that the word 'monitor 'is not interchangeable with the word 'score', with 'monitor 'referring to daily health observations and 'score 'referring to the use of the score sheets. Details about who will monitor and score on the animals should be included. The frequency and duration of both general daily monitoring and scoring should also be included.	
Details of anaesthesia (if not being used, provide justification)	

If anaesthesia (e.g. general, sedation, local or topical) is being used, please provide relevant details of agents to be used here. If anaesthesia is not being used, justification must be provided.
Details of analgesia (if not being used, provide justification)
Provide details of analgesia including dosing regimens. If analgesia is not being used, justification must be provided.
Other than analgesia and anaesthesia, list all other refinements that will be applied to this procedure (refinement is a legal requirement).
Other than pain-relieving methods and anaesthesia, additional procedure-specific refinements should be outlined. These refinements do not need to be novel refinements devised specifically for this project (e.g. provision of heat to avoid hypothermia associated with anaesthesia). It is important to include refinements which have already been identified through prior experience or literature research. Please note that refinements are a legal requirement. Entries of 'not applicable' are not acceptable.
What is the fate of the animals at the end of the procedure?
Indicate the fate of the animals at the end of the procedure, which could be • **Kept alive at the establishment**. Note that any subsequent re-use must be authorised in the relevant project authorisaton. • **Setting free to the wild or by rehoming**. Specify below the particular circumstances when animals may be set free to the wild or re-homed and detail how the qualifying criteria set out in grand-ducal regulation of the 11 January 2013 for the protection of animals used for scientific purposes will be met. • **Euthanasia,** please fulfil the next section

If the fate is euthanasia, what is the method?	Select the method of euthanasia to be used at the end of the procedure from the drop-down list. If the method is not an approved method as per Annex IV to Directive 2010/63/EU, provide justification for the use of this method. I
If this method is not an approved Annex IV euthanasia method (see Directive 2010/63/EU), provide justification:	

Note 1: If the new procedures require a new species/strain, please complete Section C6 of this form.

Note 2: Please complete Sections C7 and D of this form.

C6: AMENDMENT TO SPECIES/STRAINS

If the amendment involves the inclusion of a new species or strain, the relevant fields should be completed in Section C6.

C7: INCREASE IN TOTAL ANIMAL NUMBERS

If the number of animal used in the experiment increased as a result of the amendment, all fields in Section C7 must be completed. Please note that if the increase in animal numbers relates to multiple species, a breakdown per species is required.

D: THE 3Rs

Section D must be completed if there have been amendments to experiments, addition of new experiments, and/or an increase in animal numbers or change to the experimental design.

The Ministry of Agriculture, Viticulture and rural Development is dedicated to achieving:
- **Replacement**: a scientifically satisfactory method or testing strategy, not involving the use of live animals, instead of a procedure. Where replacement is not possible, animal use must only be permitted where justified and where the expected benefits outweigh the potential adverse effects.
- **Reduction**: Appropriately designed and analyzed animal experiments that are robust and reproducible, and truly add to the knowledge base.
- **Refinement**: refinement of breeding, accommodation and care of animals used for scientific purposes, as well as the refinement of methods used in procedures, through eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals.

When providing a justification for the numbers of animals to be used, include any statistical parameters used to calculate the sample group size. If an experienced statistician has been consulted, details of their level of involvement should be provided, for example, if they were involved at the initial stages of designing the project through to submitting the application to the Ministry of Agriculture, Viticulture and Rural Development, or if it was approved by a statistician as part of the local ethics committee review. The NC3Rs website is an excellent resource to assist in optimal experimental design and includes information on experimental design supports such as the ARRIVE Guidelines and the Experimental Design Assistant (EDA). These resources should be consulted in the design phase of all studies.

5. UPDATE TO NON-TECHNICAL PROJECT SUMMARY

There is no requirement to submit an updated non-technical project summary when applying for a project amendment. If an update is required, the Administration of Veterinary Services will ask for the updated NTPS.

6. DECLARATION AND UNDERTAKING

The declaration and undertaking section must be signed by the project leader (or the proposed project leader in the case of a new project leader being proposed), and the person responsible for ensuring compliance with the provisions of the Grand-Ducal Regulation of the 11 January 2013. In the event of the project amendment being granted, by signing the declaration and undertaking, all persons are assuming the responsibility for the overall implementation and compliance of the project with the legislation and with respect to fulfilment of the conditions and obligations as set out in the declaration and undertaking. They are also confirming they will comply with any conditions, which may be imposed in the authorisation itself, in the event that it is granted.

7. MAKING AN APPLICATION

An application for an amendment or renewal of a project authorisation must consist of a completed project amendment/renewal application form. Signed copies of all application forms must be submitted to the Ministry of Agriculture, Viticulture and Rural Development. In addition, any relevant associated documentation must be included. The necessary documentation for each category of amendments is outlined in the relevant sections of this guide.

The Ministry of Agriculture, Viticulture and Rural Development requests that applications and their accompanying documents are properly documented. Each document should begin with the original project authorisation number.