Animal Protocol Applications to the Austrian Authorities

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General Introduction

Please be aware that this information is for Austrian applications ONLY. Although the EU Directive on the protection of animals used for scientific purposes (2010/63/EU) is in place, laws are still differing between EU countries.

Although I am providing this information in English to make sure that all Austrian scientists, no matter their origin, can benefit, all documents have to be filled in German as this is the official language in Austria.

! Before submitting your application to the authorities, please contact the veterinarian / animal welfare body of your institution and discuss your project with them as most institutions have an internal control body that needs to check and approve each application before it can be submitted!

Where to submit

In Austria, you have to submit your animal protocol to different authorities, depending on the nature of your institution and your institution's location:

If you work at a University or non-university research institute you have to apply to the federal government.

If you work for a company, association, or else, you need to apply to your local state authorities.

- Vienna (online submission only)
- Styria
- Upper Austria, Lower Austria, Burgenland, Carinthia, Salzburg, Tyrol, Vorarlberg: no information available. If you know anything about applications in these states, please let me know!

Which documents to submit

Depending on the authority that is responsible for your application, documents look slightly different, so please download all needed documents from the corresponding website (see above). In general, you will need the following:

Three documents are mandatory for each animal protocol independent on the nature of your experiments:

- Antrag auf Genehmigung eines Projekts (Application for approval of a project)
- Projektbeschreibung (project description)
- Nichttechnische Projektzusammenfassung (non-technical summary)

If you are planning to perform animal experiments with a genetically modified animal model, you additionally need to submit this document:

- Beilageblatt "Beschreibung von genetisch veränderten Tierlinien"

If you established a new research group and you/your supervisor did never apply for an animal protocol at this institution before, you/your supervisor will also need to apply to become a licensed principal investigator:

- Antrag auf Genehmigung als Projektleiter/in

Only scientists with a completed academic education and/or advanced trainings and courses in relevant fields are able to apply.

Which information you need

Before you start filling these documents, you need to plan your project in detail:

What is your study setup? Decide, which animals you want to use? Age? Sex? Number? Controls?

How do you manipulate these animals? Treatment? Surgery? Behavioral tests? Food deprivation? Single/Group housing? Environmental enrichment? Do you need to evaluate clinical signs?

Detailed protocols of everything you are planning to do, for example:

- To perform surgery, you need to know which anesthesia and analgesic you are going to apply. How do you close the surgery wound? How do you work as sterile as possible? If you need help with such planning, contact the veterinarian of your institution.
- For your behavioral tests you need detailed protocols: How many trials are planned? If you give electric foot shocks, how strong and how long are they? How often do you plan to give them?
- If you want to treat your animals with a compound, you need to know the planned volume, concentration, injection route etc. For more information about acceptable volumes by route and species please see the GV-SOLAS Fachinformation. What is already known about this compound? Effects and side effects? Which vehicle are you going to use and why? For more information about the most used vehicles and tolerability, I recommend Gad et al., 2016.

Severity evaluation

Additionally, you need to evaluate the severity of your animal study. You need to select from four categories:

1. **No recovery of vital functions:** This applies if your entire animal study is performed under general anesthesia from which the animal will never wake up.
2. **Mild:** Animal experiments where it is expected that animals experience only short and mild pain, anxiety or suffering as well as experiments without impairment of wellbeing and general health.
3. **Moderate:** Animal experiments where it is expected that animals experience only short and moderate pain, moderate anxiety or moderate suffering or long-lasting mild pain as well as experiments with moderate impairment of wellbeing and general health.
4. **Severe:** Animal experiments where it is expected that animals experience severe pain, severe anxiety or severe suffering or long-lasting moderate pain, moderate suffering or anxiety as well as experiments with severe impairment of wellbeing and general health.

You first have to assign a category for each manipulation you are performing in your study, like treatment, compound, each behavioral test, single housing and so on. Once done you have to evaluate the cumulative severity of your project by “summing up” the categories of all your tests. This “summing up” is quite subjective and usually it corresponds to the highest category given in the individual assignments or one category above.

As this severity evaluation is quite confusing to many people, I would recommend the following official pages that provide examples to each category:

- Zuordnung von Schweregraden zu Tierversuchen (Styrian government)
- Caring for animals -aiming for better science (EU)
- Schweregradbeurteilung von Verhaltensexperimenten mit Mäusen und Ratten (Austrian government)

3Rs

In the application form, you also need to discuss the 3Rs related to your animal study:

Replacement: Why do you have to perform the planned animal experiment and why are you not using alternative methods (in chimico, in vitro, in silico, omics)?

Reduction: How do you ensure that you use the least possible number of animals without risking to lose too much statistical power?

Refinement: How do you improve the animals' wellbeing? How can you reduce pain, suffering and stress to the study animals? This can include:

- Choice of species (using mice instead of non-human primates)
- Group housing
- Environmental enrichment
- Training level of people performing the experiments

You can find more information about the 3Rs here

- EU
- The RepRefRed Society (Austria)
- British National Centre for 3Rs

Statistical evaluation of group sizes

Why are you using the planned group size? When planning an experiment including behavioral tests you should probably choose a larger group size compared to a pharmacokinetic study where you inject the animal only once with a compound, as the variability of behavioral tests is much higher. To be able to argue choosing the group size properly, it is mandatory to perform a statistical power analysis with historic data of a comparable study whenever possible.

Free online tools to perform a power analysis:

- Power and Sample size online tool (Nerds from Atlanta)
- Power & Sample size calculator online tool (University of Iowa)
- G*Power free program for download (Heinrich Heine University Düsseldorf)

As this statistical evaluation is quite confusing to many scientists that are not naturally-born statistician, I strongly recommend to get help from someone experienced with such analyses or even ask a professional statistician.

Literature search

Finally, you need to perform a literature search to validate that your study does not repeat an already existing study. You may to do this by using different sources, like PubMed, Google scholar or any other appropriate knowledge data base.

After you collected and prepared all information as described above, it is now time to fill the documents. We will start with the “Projektbeschreibung” (project description) because afterwards we can easily fill the remaining documents using information that we already provided in the project description.

Projektbeschreibung (project description)

The project description needs to contain the below listed points a-j. This structure is mandatory.

Add a project title describing your planned animal study

a. Importance and justification of your project, project goals

- Describe the question / hypothesis behind your project. What is the goal of your study and why? What is the clinical relevance of the expected results?
- Provide an introductory text to your study. What makes your study state-of-the-art? What makes your study indispensable? Add references and information about already performed *in vitro* and *in vivo* studies (including the GZ number) and related own publications. Are you cooperating with other research groups on this project?
- It is also helpful when your introductory text answers the following questions:
    - Who profits from your study? Humans, animals, environment?
    - How large is the benefit of the study for other scientific or didactic purposes?
    - How high is the significance of the study compared to international research?
    - Is it possible to transfer results of your study to other animal species or humans?
    - Do the results of this study lead to scientific, practical, or didactic value?
    - What is the likelihood that the expected benefit will be generated in this study?

b. Justification for the use of planned animals, including species, number, origin and age

- Why is the species you are planning to use the best model of choice? Describe the genetic background, genetic modifications
- What is the main readout of your study and what is the unit of measuring?
- What is the significance level of the planned statistical analysis (usually its 5 % that corresponds to $p<0.05$)?
- What is the statistical power of this analysis (should be at least 90%)?
- Relevant difference between means (effect size)
- Assumed standard deviation within groups (Reference to published data, if not available provide own preliminary data or rough estimate)
- If you analyze more than two groups: Correction for occurrence of false positives by using *post hoc* tests for multiple comparisons (alternatively, manual correction by dividing the level of significance by number of planned comparisons; for example, for 6 pairwise comparisons within 4 groups: significance level = 5 / 6 = 0.83 %).
- What is the origin of your animals? Are they bred in-house or by another breeding facility?
- How old are the animals at start of the study?

c. Experimental strategies and statistical evaluations

(Please be aware that the statistical evaluation of group sizes as described in b. needs to be presented in c. if you submit your application to the Styrian government.)

- Describe your project in detail. What are you planning to do and when?

- If your study is very detailed, it is useful to provide a table with groups and differences between groups (test vs. control groups, group size etc.)
- If your study runs during extended time, it is useful to add a time schedule, similar to a Gantt chart
- How do you prepare animals for the tests?
- Describe each planned treatment, test, method and so on in detail
- Describe the method of genotyping if applicable, how do you collect tissue for genotyping? How many animals do you have to breed and genotype to yield a sufficient number of animals with proper genotype for your study? If you need to breed surplus animals to yield the required animals of the proper genotype, it is helpful to add a schematic drawing of your breeding scheme.
- Perform severity evaluation as described above
- Which medical measures are you taking?

d. Use of anesthesia, analgesia and other pain reducing agents

- Do you need to anesthetize your study animals? If yes, how do you do it? Describe the method in detail.
- Do the study animals require analgesia? If yes, what substance are you using? Which concentration? How often? How do you evaluate if animals are in pain?
- Are you using any other treatment that will reduce animals pain perception?

e. Use of painless endpoints and termination criteria

- For each treatment, test, method a.s.o. that you are using you have to define termination criteria. What happens at this point? Are you going to treat or kill the animal?
- Add general termination criteria
- If you are performing a survival analysis or you use animals that will develop a severe phenotype during the course of the study, you will need to evaluate clinical signs. In this case you should add a clinical signs sheet to the applications that provides an animal model-specific scoring system clearly defining at which point the animals have to be euthanized to reduce suffering.

f. Way of killing (if applicable)

- If you are planning to kill your animals at the end of the study, you need to explain how you are going to do it. Describe the method in detail and explain why you are using this method if it is not a standard method like overdose of anesthetic.
- It can be useful to shortly explain which analyses you are planning to perform with the collected tissues as this can further support the indispensability of the animal experiment(s)

g. Information about the 3Rs

- Discuss the 3Rs as related to your project (see above)

h. Description of housing and care, description of whereabouts of animals if not killed after completion of study

- How are animals housed? Type of cage? Litter, food, water. How often is it checked/changed? Special diets?

- Room temperature, humidity
- Light-dark cycle
- Hygiene measures
- Safety measures
- Single / group housing
- Enrichment
- Quarantine
- Veterinary care
- Periodical health checks
- Special care needs depending on model
- What happens with your animals if you are not planning to kill them at the end of the study? How do you prevent a repeated use of the animals for other experiments? If you are planning to use the study animals for an additional study that is not described in this application, you have to explain why.

This part does mostly not change much as long as animals are housed in the same animal facility. Ask a colleague who already applied for an animal protocol if she/he can provide it to you.

i. Reduction, Prevention and mitigation of any suffering from birth to death

Describe in detail, which severity score you are selecting for each experimental part of your study and why. For example, if you inject a transgenic animal with a compound, you have to evaluate the severity of the transgene, the injection procedure, the compound and the vehicle. Do not forget the severity score of genotyping or shipment (if your animals are not bred in your facility). Specify how strong (mild, moderate or severe) the pain, stress and suffering for the animal will be for each experimental part and in which severity score this evaluation results.
At the end, describe the severity of the cumulative suffering for the animals by summing up the severity scores to a total score (See above for details).

j. Prevention of not justifiable double performance of animal experiments

If your study does not repeat an already performed animal experiment you need to explain, how you know this. Which sources did you use for a literature search to evaluate if the experiment(s) were already performed by others?
If your study does repeat an already performed animal experiment you need to explain, why you are planning to repeat this study. If possible, cite the original study. A possible reason for repeating an already performed experiment could be to include it as reference or control treatment (see above for details).

Once you completed the project description, it is easy to fill all remaining documents:

Nicht-technische Projektbeschreibung (non-technical summary)

The template for this document was changed by the Austrian authorities as of January 2021. So please make sure that you are using the new template!

! This document will be published online by the responsible authority, so make sure that it does not contain any personal information that would allow to track the document back to you or your institution. This includes detailed information about the animal model used or similar detailed information!

The whole document should be written using language that is understandable for non-scientists as it is published online for lay persons.

- Add the title of your project as already used in the project description.
- Add the duration of your project (≤ 30 months).
- Add 1-5 keywords (≤ 50 characters).
- Describe the goals of your project by choosing from the drop-down menu and additionally describing it (see project description a.; ≤ 2500 characters). The drop-down menu contains a list of purposes that you will find again in the **Application for approval of a project**.
- Describe the benefit of your project (see project description a.; ≤ 2500 characters)
- a) describe the planned animal experiments (injections, surgeries, behavioral tests; see project description c.; ≤ 2500 characters).
  b) Describe the expected harm to the animals like pain, stress, weight loss, motor deficits (see project description i. without mentioning the severity score; ≤ 2500 characters).
- Harm to the animals. If you click on the title, a table opens that you have to fill by choosing the animal species you are planning to use and how many animals per species are expected to experience which severity score. Numbers are estimates.
- If you are planning not to euthanize your animals at the end of the study, you need to add information about the whereabout of the animals after completion of the study. When you click on the title a table opens that you have to fill by adding the species and the number of animals per species that are expected to be
  - Reused
  - Returned to their habitat / housing
  - Privately housed

  Additionally, you need to justify the whereabouts of the animals after completion of the study (≤ 2500 characters).

- Describe the 3Rs (see project description g.; ≤ 2500 characters for each of the 3Rs).
- Explain why you chose the species and the age of the animals for the planned study.
- The last paragraph asks about the retrospective evaluation of the project. As not every study needs to be evaluated retrospectively and the decision about it is made during the assessment by the authorities, this needs to be filled by the authorities.

Antrag auf Genehmigung eines Projekts (Application for approval of a project)

1. Add the project title, as already mentioned at the start of the project description.
2. Who is the principal scientist (PI) of the planned project? If the person was already PI for a previous animal study, mention the project number (GZ) and date of approval.
   Do you plan to perform surgery in the planned project?
3. Who is the user/institution "Verwender/Einrichtung" of the project? This can be a university, institute or company. When was this "user" first approved as "user"? Mention the project number (GZ) and the date of approval.
4. Who will work with the animals of your project?
   Include all people that are in contact with the animals and their education/title: Who performs the experiments? Who cares for the animals (animal keeper)? Who kills the animals (if applicable)?
5. What is the purpose of the planned animal study? Choose one or more from the provided list.
6. List animal species, origin, number and transgenicity.
   - Are you planning to use animals that are not explicitly bred for the use in animal experiments?
   - Are you planning to use methods of killing that are not listed in § 20 TVV 2012?
   - Are you planning to use wild life animals, endangered species or non-human primates?
7. What is the cumulative severity score of your planned animal study?
8. Project duration start and end of the study like: 03/2021-02/2023.
   Please be aware that the maximum length of a project is 5 years.
9. In case your study needs to be evaluated retrospectively after completion of the project, you need to be able to provide relevant study documents. If your study has a severity score of "severe" you need to provide documents within two months after study completion. Is the severity score "moderate" or below, you need to provide documents within one month after study completion. The decision if your study needs to be evaluated retrospectively at all will be mentioned in the study assessment provided by the authorities.
10. Are you performing the animal study to adhere to regulatory requirements?
    If yes: according to which regulations? If you already wrote about it in the project description, reference to the corresponding page.
    If no: Fill points 10B-O. Choose from the provided selections and justify your response to the following questions:
    - B How large is the benefit of the study for other scientific or didactic purposes?
    - C Who profits from your study? Humans, animals, environment?
    - D-E What is the significance of the study compared to international research?
    - I Is it possible to transfer results of your study to other animal species or humans?
    - J Do the results of this study generate scientific, practical or didactic value?
    - K What is the likelihood that the expected benefit will be generated in this study?

    While writing the introduction of the **project description**, I mentioned that it would be good to provide answers to all these questions. If you did, you can now reference to the corresponding page of the project description (a.).

F-H If your study relates to one or more of the 3Rs, mention the value here. (see project description a.)

L Are you planning to publish the results of the planned animal study? If not, you have to justify why.

M What percentage of animals in your study are categorized to each severity score? Please be aware, that even if your whole study has a severity score of “severe” your control groups might just have a score of “moderate” or “mild”. (see project description i.)

N Is it expected that animals experience severe pain, severe anxiety or severe suffering or long-lasting moderate pain that will be long-lasting and that cannot be alleviated?

Please be aware, that a severity score of “severe” does not automatically mean that you have to choose “yes” here. The important part of this question is “long-lasting and cannot be alleviated”. If your study still belongs to this category, you have to scientifically justify why this animal study is necessary. If there is no justification for it, your study will not be approved and the planned project is prohibited.

11. The application needs to be signed by the PI and the “user” (institutional official)

Beilageblatt "Beschreibung von genetisch veränderten Tierlinien"

For each genetically modified animal model you are planning to use in your animal study you need to fill an extra "Beilageblatt". You therefore have to give the document a number.

1 Add the Title of your planned study as mentioned in the project description and application.

2 Are you using a genetically altered model or an induced/naturally occurring mutation?
   a) Provide a reference for your model – who published the model first?
      Also provide a unique ID number of the model (e.g. MGI ID, order ID from commercial vendor)
   b) Are you performing the experiments with transgenic vertebrates for biomedical or for evolutionary biology purposes?
   c) Describe the modified gene and its function
   d) Describe the animals' phenotype specifically relevant for suffering (genetic background, phenotype of homozygous/heterozygous/hemizygous animals, age-dependent phenotype
   e) Which methods are you planning to use to reduce suffering and animal numbers (breeding plan, special requirements for care and housing, termination criteria).

3 What is the severity of suffering of the animal model? Is the model burdened by the genetic modification or not? You need to proof your choice by publications. If you are planning to evaluate the severity of suffering in the here proposed study, you need to add a check list of line-specific observations to your application. The observations should include measurements of expected phenotypes.

4 What is the severity score of the animal model caused by the genetic modification? Here it is explicitly asked for the score caused by the genetic modification, and not for the overall severity score the animals are exposed to during the whole study.

5 Are you planning to genotype your animals? If yes, you need to describe the following points in your project description (c.):
      i. Planned method of tissue collection (taking tail tips is already an animal experiment, as it is not necessary for the identification of the animal. In this case all animals you are genotyping are automatically considered study animals! If you use ear tissue collected during ear marking or mouth swaps, this is not considered an animal experiment)
      ii. Animal numbers to be genotyped
      iii. Severity score of genotyping

The description of the four documents gives you an overview how to fill the most important documents for an application and should be sufficient for most applications.

Additionally, there are further institution-specific documents or a document if you need to change aspects of your already approved application. Please be aware that only a change of the following aspects is possible:

- Change of project duration
- Change of animal number
- Change of methods
- Change of experiment location
- Change of PI
- Change of personnel performing the experiments

! If you change too much resulting in a higher severity score of your study, it is advisable to send a whole new application!

Final Report:

The content of the final report strongly depends on the nature of your animal study, specifically on the severity score. Which information such a report needs to contain will be listed in the assessment provided in the approval letter of the authorities.

Please read this assessment in detail before you start your experiments to make sure you are evaluating the health status of your animals accordingly and you are collecting all required information during the experiment.

If you have any questions or concerns relating this guide, please feel free to contact me!

Disclaimer of liability: Please be aware that documents and requirements can change. So please check the provided online sources for changes. BioDoks cannot be held liable for any incorrect information that might be displayed in this guide.

Useful additional information

- Austrian Tierversuchsgesetz 2012
- Information brochure on animal experiments by VetMed Wien
- Manual for applying for animal protocols by MedUni Wien
- GV-Solas homepage
- Guidance documents of the EU

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