Guidelines to the project licence application form

In these guidelines, the three documents that constitute the project licence application form will be discussed:

- Project licence application for animal procedures (contact details institute and applicant);
- Non-technical summary (for the general public)
- Project proposal (description of project)

Project licence application for animal procedures

This form should be used to:

- submit an **application for a project licence**.
- submit an **amendment to a project licence**. An amendment should be submitted when the proposed alterations will adversely affect the welfare of the animals or affect the basic principles of the project licence.
- notify the Central Authority for Scientific Procedures on Animals (CCD) of alterations to a project. It is sufficient to notify the CCD when the proposed alterations do not adversely affect the welfare of the animals.

You submit your application via our digital form at https://mijn.rvo.nl/dierproeven.

1. Details applicant

1.1 Approval number from the 'Netherlands Food and Consumer Product Safety Authority'
In the Netherlands, animal procedures may only be performed in establishments that are authorised by the competent authorities. Licences are issued by the 'Netherlands Food and Consumer Product Safety Authority', also known as the 'NVWA'. These licenced establishments have also received an approval number from the 'Netherlands Food and Consumer Product Safety Authority'. This approval number should be provided here. In Dutch, the approval number is known as *'deelnemernummer NVWA'*. If the project is a collaboration between multiple establishments, only the approval number of the establishment responsible for this project should be provided.

1.2 Type of application
Select the appropriate category (new application, amendment, or notification). For more information on amendments and notifications, see beleidsregels 'Meldingen in het kader van een projectvergunning', the corresponding guidelines, and the guidelines on amendments (toelichting Wijzigingsaanvragen). For amendments and notifications, please provide the AVD number assigned to the original application.

1.3 Contact details of the establishment licence holder
If the establishment licence holder is a legal entity, the name of the natural person who is mandated to act on behalf of the establishment licence holder should be provided here. This person is also known as the portfolio holder. Also include the email address of the portfolio holder or his/her contact person. The portfolio holder, in turn, can authorise another person to act on his/her behalf. In this case, a mandate has to be enclosed with the project application (see 1.8). The mandated person will, in addition to the responsible researcher, receive all correspondence on the project licence application.

1.4 Responsible researcher (project leader/principle investigator)
This person is responsible for writing the project licence application and performing the procedures according to the project licence. In addition, this person should meet the requirements mentioned in article 9 of the Animal Procedures Act. This person will ensure that when the project is not performed

in accordance with its project licence, appropriate correcting measures will be taken which will be registered.

If the responsible researcher resides outside the Netherlands, he or she must have an (employment) agreement with the institution where the research will be conducted.

1.5 Acting responsible researcher
A second responsible researcher can be appointed, and he/she must also meet the requirements mentioned in article 9 of the Animal Procedures Act.

1.6 Person responsible for conducting the project
This person ensures that when the project is not conducted in accordance with the project licence, appropriate correcting measures will be taken which will be registered.
In many cases, this will be the same person as mentioned in 1.4. However, the establishment licence holder or responsible researcher can delegate these responsibilities to another experienced person. This person should occupy a position that allows him/her to exercise these responsibilities.

1.7 Details Animal Welfare Body
Contact details of the Animal Welfare Body can be included here. If these details are included here, the Animal Welfare Body will also receive all correspondence on the project licence application.

1.8 Mandate by establishment licence holder
The establishment licence holder and the portfolio holder can mandate an employee or a third party to apply for the project licence. The mandate form (Melding Machtiging) should be filled out completely and enclosed with the project licence application. This mandated person can be authorised for one application or indefinitely. Once a mandate has been issued it is not necessary to include a new mandate form with each application.

2. About your application

2.1 Amendment to a project licence
If a proposed alteration alters the basic principles of the licence, adversely affects the welfare of the animals or requires more animals, an amendment must be submitted to the CCD for approval. For other alterations for which an amendment needs to be submitted, see the beleidsregels 'Meldingen in het kader van een projectvergunning' and the corresponding guidelines. The details of the proposed alterations should be described in the project licence application form. In addition, a justification for the proposed alterations has to be provided. Furthermore, those sections in the project proposal and the appendix Description animal procedures that are relevant for the amendment should be filled out. If the proposed alterations lead to an increase in the number of animals, not only fill out question B 'the animals', but also questions A' Experimental approach and primary outcome parameter' and G 'Replacement, reduction, refinement'. If the proposed alterations lead to an increase in the classification of the severity of the procedures due to the changes in the animal procedures, not only fill out question A' Experimental approach and primary outcome parameter' and F 'Classification of severity of procedure', but also D 'Pain and compromised animal welfare', E 'Humane endpoints' and G 'Replacement, reduction, refinement'. You must use the latest version of your project licence application. If you make alterations to the original project licence application, please make these alterations visible for the CCD by, for example, using bold print or a different colour. If you wish, you may also include a separate letter to the application in which the alterations are described and justified. For more information on submitting amendments, please see the Guidelines on amendments.

The non-technical summary (NTS) should be updated by the applicant. It is not allowed to submit an empty NTS form in which only the amendments are depicted. The altered procedures may not be performed unless the CCD has authorised the amendments. The altered NTS will not be published until the CCD has authorised the amendments.

If you are submitting a follow-up application where the decision of the previous study includes a requirement for annual feedback to the CCD, you can indicate this here. The annual feedback from the previous study will then be sent to the DEC for ethical review when the CCD asks the DEC for advice on your follow-up study.

2.2 Notifications
If a proposed alteration does not adversely affect or even positively affects the welfare of the animals and does not require more animals, it is sufficient to notify the CCD of these alterations. Although no authorisation from the CCD is required, the CCD has to be notified in a timely manner, within two months after implementing the alterations, to ensure that the project does not deviate from its licence. To notify the CCD, the project licence application form can be used. Multiple notifications may be combined. If notifications are combined, for each licenced project a different project licence application form should be used. Please mention the title of the project, the AVD number, and the date of implementing the alterations. The application form has to be signed by the port folio holder or mandated person. In addition, the details of the implemented alterations should be described in the project licence application form. Furthermore, a justification for the alterations has to be provided. The opinion of the Animal Welfare Body on the effect of the alterations on the welfare of the animals should also be included in the application. If you wish, you may also include a separate letter to the application in which the alterations are described and justified and the opinion of the Animal Welfare Body is mentioned. If the original NTS is no longer representative of the application due to the implementation of alterations, an updated NTS should be provided. The altered NTS will be published on the website of the CCD.

For more information of notifications, see the beleidsregels 'Meldingen in het kader van een projectvergunning' and the corresponding guidelines.

3. About your project

3.1 Start date and end date
Keep in mind that the actual start date depends on the time required for the evaluation of the project licence application. Project authorisations may be granted for a period of maximally 5 years.

3.2 Title project
Use a descriptive and unique title which covers the content of the project application. The title cannot be changed during duration of a project.

3.3 Title non-technical summary
The title of the non-technical summary should be provided here. This title should be informative, with the public able to understand all parts. This title may be different from the title provided in 3.2.

3.4 Animal experiments committee (DEC)
Provide here the name of the preferred animal experiments committee.

4. Payment details

4.1 and 4.2 Payment details
The administrative expenses for the evaluation of the project licence application or amendment must be paid by the applicant. For this, you will receive an invoice from the CCD. If the billing address is different than the postal address mentioned at 1.3, provide here the billing address. If an order ID is required for payment of the invoice, such a number can be mentioned at 4.2.

5. Checklist appendices

5.1 List of appendices
The appendices which will be enclosed with the licence application form should be listed here. Appendices that are non-compulsory should only be included when they are essential to the evaluation and authorisation of the project. Please note that it should be possible to understand the project licence application without any additional appendices. Before submitting the application, ensure that all necessary appendices have been enclosed.

6. Signature

6.1 Signature
The licence application form must be digitally signed by the establishment licence holder or the mandated person. The person who signs the licence application form will ensure that the

administrative expenses are paid within the defined payment period. In addition, the person who signs the licence application form promises that the animals will be housed, cared for and treated according to legal requirements. Furthermore, he/she will ensure that the staff are adequately educated, competent and continuously trained, and that they are supervised until they have demonstrated the requisite competence.

Non-technical summary

The Non-Technical Summary (NTS) should be written in Dutch. For this purpose, the form prepared by the European Commission (Excel file) need to be used. Once you indicate in this document (from version 1.1) that the NTS is in Dutch, the questions and explanatory text are automatically displayed in Dutch.

The responsible researcher is responsible for drafting the NTS and should involve the animal welfare body (AWB). When drafting it, keep the target audience, the general public, in mind and adapt language accordingly. Avoid veiled language, technical details, abbreviations and jargon. Be unambiguous and understandable. Provide only requested and relevant information. Ensure that the NTS is consistent in all sections with the project description, including appendices, and does not contain references and/or names to the institution licensee or the investigator in charge. The submitted NTS should be suitable for publication. The NTS will not be published until the project license application is approved by the CCD.

For more information on writing the NTS, See the "NTS guidance document for users" on the CCD website.

Project proposal

The project proposal constitutes the main document of the project licence application. It must include all information that is required for the CCD to perform the legally required ethical evaluation and project authorisation. The purpose of the project proposal is to inform the members of both the DEC and the CCD of the proposed procedures and the project's scientific or social relevance in a realistic and understandable manner. The evaluation of the ethical acceptability of the proposed procedures requires a justification of the choices that were made during the design of the project. It is therefore essential to present a detailed plan of investigation in which the relevance of the project is highlighted. In addition, the project's proposal should explain to what extent the project complies with the legislation in force and meets the requirements for Replacement, Reduction and Refinement of the use of animals in procedures, and how the "no unless" principle is guaranteed.

With regard to the formulation of the project proposal, it should be noted that experts from disciplines other than those covered by the project proposal are also involved in its evaluation and review.

Readability
It should be feasible to understand the project licence application without any additional information. Appendices that are not compulsory should only be enclosed with the project licence application when they are essential to the evaluation and authorisation of the project. Each of these appendices should be cited in the text. Moreover, the relevance/importance of the appendices should be discussed.

Project
The term 'project' refers to a coherent program in which at least one procedure is performed using at least one animal of at least one species. The individual procedures should be correlated and should serve a common, well-defined and verifiable purpose that can be achieved within the authorisation period.

A project can be part of a bigger program (for example the program of a department or institute) in which experimental procedures will be performed that do not involve the use of animals. In the project proposal, you should focus on the procedures in which animals will be used. Other procedures should only be included when they are important for the execution, order or coherence of the procedures. For more information on what is meant by the term 'project', see the practical guidance 'Invulling definitie project' on the website of the CCD.

1. General information

Provide general information on the project, including a descriptive and unique title which completely covers the content of the project licence application. This information should be identical to the information provided in the project licence application form.

2. Categories

The project should be designed to serve a specific purpose. To answer the question of which purpose will be served, at least one category of study objectives must be selected here. These objectives should be identical to those selected in the non-technical summary. More than one category may be appropriate. See Article 10.1 of Implementing Decision EU/2020/569 for definitions of the different categories. Or the working document on genetically altered animals from the European Commission on the CCD website.

3. General description of the project

3.1 Background
Describe the (inter)national background, context of and motivation for the hypotheses and objectives of this project. These hypotheses and objectives will be described in Section 3.2 of the project proposal. Each of the objectives selected in Section 2 should be discussed here. In terms of which information will be required for evaluation of the project, this is dependent on the selected category. For orientation, some examples of topics are given below.

For fundamental and translational research:

- Describe the current situation in your research area (scientific literature, your own research). You may also refer to a few key publications (summarise the relevant information in these publications).
- Describe the preliminary results, considerations and scientific hypotheses on which this project is based.
- What preliminary work ((systematic) review, previous research, alternative (*in vitro, in silico*) testing) has already been done so that the "no unless" principle is guaranteed?
- To which extent will the project contribute to the progress in your research area?
  If the current project licence application is a continuation of a previously authorised project, information should be provided on the original project. When relevant, also mention the results obtained from previous research.

In the case of research to protect the environment in the interest of human or animal health or welfare:

- How does scientific use of animals relate to the protection of the environment in the interest of human or animal health or welfare?

In the case of research aimed at preserving the species:

- What is the current situation regarding the species to be conserved (those used in procedures and/or others)?
- In what ways are animals currently protected?
- In what ways will the project promote species conservation?

For legally required research:

- Explain on which legal requirements (proposed use and/or market authorisation) the proposed research is considered necessary.
- List the admission authorities which demand the tests.
- Explain which legislation will be satisfied by these procedures (see the Implementing decision 2012/707/EU for legislative requirements).
- What is the ultimate purpose and/or added value of the substance to be tested compared to substances already in use, for example?
- What preliminary work ((systematic) review, previous research, alternative (*in vitro, in silico)* testing) has already been done so that the "no unless" principle is safeguarded?
- What criteria must be met before a particular substance is tested in an animal test?

For routine production:

- Describe for which applications the product(s) are necessary.
- Provide information on the expected demand for this/these product/products.
- What is the ultimate purpose and/or added value of the substance to be tested compared to substances already in use?
- What preliminary work ((systematic) review, previous research, alternative (*in vitro, in silico*) testing) has already been done so that the "no unless" principle is safeguarded?
- What criteria must be met before a particular substance is tested in an animal test?

For higher education or training:

- Explain why this project should be part of the educational program and provide information on the learning targets.
- Explain how this project is embedded in the educational program.

For projects focussed on animal husbandry

- Describe the current policy of the government and /or the livestock farming sector with respect to improving sustainability of livestock farming. Sustainability may include improving animal welfare and health, public health, nature, and the environment. Limit your answer to the livestock farming systems your project focuses on.
- Describe how your project relates to above mentioned policy.

- Describe how the knowledge obtained in this project may be applied to improving animal welfare and/or other aspects of sustainability
- If applicable, describe how the knowledge obtained in this project may be used to replace, reduce or refine animal procedures in the future.

If the application concerns a follow-up project, include information on the results of the previous project(s) (also mention the AVD number(s) of the previous project(s)). For the evaluation of applications for follow-up projects, it is essential to know the current status of the research area and how the follow-up project will contribute to the progress in your research area. For the evaluation of the feasibility of the follow-up project, it is essential know the achievements of the previous project. The CCD will not perform a retrospective assessment. You therefore do not need to include information on numbers of used animals. New discoveries leading to optimisation of the 3Rs can be mentioned in section G of the appendix Description of animal procedures. If a systematic review has been performed, the results can be described here.

3.2 Purpose

3.2.1 Immediate goal and ultimate goal
Describe both the immediate goal (main objective) and the ultimate goal. Explain what you are aiming to achieve, confirm, investigate, produce, test or obtain by undertaking this project. While the main objective should be clear, realistic and achievable within the duration of the project, the ultimate goal does not need to be feasible within the duration of the project.

The project can be composed of multiple procedures that each serve a specific subobjective. These subobjectives should also be mentioned here. The individual procedures have to be described in Section 3.4 (research strategy). For all individual components, the relationship to the main objective should be clear.

If there are secondary objectives (such as the development of animal free models and refined animal procedures or improving sustainability of livestock farming), this should be mentioned here.

3.2.2 Feasibility
To justify the project's feasibility, the following aspects should be discussed: the availability of required expertise, the infrastructure and the design of the research plan. The availability of the expertise required to both adhere to the principles of replacement, reduction and refinement (3Rs) and to prevent negative effects on animals, humans and the environment should also be discussed. It is not necessary to describe which methods for replacement, reduction and refinement will be applied in the experimental design. These methods should be described in the Appendices: Description of animal procedures.

3.2.3 Other laws and regulations
Indicate whether other laws and regulations are applicable during this project. It is the responsibility of the establishment licence holder to comply with applicable legislation as it relates to research. Also, the establishment licence holder must ensure that exemptions related to the research are timely requested.

This is necessary because certain laws and regulations have precedence over the Animal Procedures Act. Examples are legislation aimed at protecting the health and welfare of animals, and the preservation of species, such as 'Wet Dieren', 'Omgevingswet', and the 'Transportverordening'. Describe the effect of these laws and regulations on the feasibility of the immediate goal and the welfare of the animals. Indicate whether an exemption is required and you have obtained or applied for it. If it necessary for your project to derogate from these or other types of legislation, and this is allowed, provide a justification. If a project cannot comply with all relevant laws and regulations, and exemption cannot be granted by the competent authorities, a project may not be conducted. In these situations, the immediate goal will not be feasible. If an experiment cannot be completed, animals have been used needlessly. This should be prevented.

If all relevant laws cannot be complied with, nor can an exemption be obtained from the competent authorities, the project cannot be implemented. This compromises the feasibility of the project. It may also happen that a trial has to be stopped halfway through because it is not carried out according to legislation. In such situations, animals are used unnecessarily. This should be avoided in the context of "reduction". Finally, if other legislation stipulates that animals may be exempted from

relevant legislation because animals are part of an animal experiment, the CCD should assess whether the relevant conditions are met.

Animal welfare may also be reduced as a result of compliance to other laws and regulations. For example, housing of animals under existing farming practices (according to Wet Dieren) instead of the requirements for the care and accommodation of animals according to the European Directive (2010/63/EU).

If the project aims to market products outside the EU, this should be mentioned here. If within Europe an alternative has been accepted for which animals are not required, this should also be mentioned. If, in comparison to European regulations, more animals are required to market a product, provide information on the differences in animal numbers.

3.3 Relevance

3.3.1 Scientific and social relevance
This section focusses on the importance of achieving the main objective.

To describe the *scientific relevance*, information may be provided on the importance of this project for (the progress of) the applicants' own research, the applicants' research field and/or other research fields (national and international). Instead of discussing the importance of, for example, cancer research in general, the importance of achieving this project's main objective for cancer research should be discussed.

The term *social relevance* should be interpreted broadly. To explain the social relevance of this project, information should be provided on its clinical, educational or economic significance. If applicable, the effects on protection of nature and the environment should be discussed, or the interest of citizen/animal safety in legally required research.

3.3.2 Stakeholders
Here you should indicate which parties have an interest in the execution of this project and/ or the achievement of the immediate goal. In addition, describe their specific interests. Potential stakeholders are target groups, experimental animals, the establishment licence holders, researchers and other interest groups or entities relevant to the project, such as the environment and society. It is not necessary to provide names of individual stakeholders, you can limit yourself to groups of stakeholders.

Only primary interests should be mentioned here. Interests that may be achieved in the far future do not have to be mentioned here.

If the project aims to contribute to the development of animal free models and/ or more refined, techniques, you must indicate here what this means for future research. For applications focusing on animal husbandry, the putative effects of the project on the welfare and health of the target animals and the sustainability of livestock farming should be described.

3.4 Strategy

3.4.1 Experimental strategy
This section focusses on the strategy that will be used to achieve the project's main objective.
If the project comprises multiple components, such as animal procedures and sub-objectives, the individual components should be listed, their coherence should be explained, and their relationship to the main objective discussed. When describing the coherence between the individual components, it is important to discuss whether these components are interdependent in terms of time and/or outcome. The different steps in the project can be visualised using a time line. Specify the go/ no go moments between de different components of the project and during the animal procedures. Describe, for each of the go/ no go moments, the criteria for deciding whether the procedure/project will be continued or cancelled.

For clarification, it may be helpful to apply project components that contribute to the achievement of the objective but are not animal experiments (other research methods, an educational program as a

whole, etc.). Including a schematic representation of the experimental design of your project can be illustrative and enlightening.

3.4.2 Justification of the strategy
Provide a justification regarding the choice of the strategy described under 3.4.1. A justification for the order of the animal procedures, the different phases in the project and the coherence should be included. In addition, describe whether the selected strategy affects the number of animals and/ or animal welfare. Information about the individual animal procedures should be provided at the corresponding appendix Description animal procedures.

3.4.3 Type of animal procedures
The term 'type of animal procedure' refers to a specific combination of experimental procedures for which at least one animal of a certain species is used. All procedures that an individual animal undergoes from the start of the experiment until the end may be described in one appendix Description animal procedures. However, in case the experiment consists of many different procedures and variations of procedures, it is allowed to use multiple Appendices Description animal procedures. If an individual animal is used in multiple appendices, it is important to mention this in these appendices. described in one appendix Description animal procedures.

In 1 appendix Description animal procedures, all animals undergo similar procedures. The effects of the procedures on animal welfare should therefore be similar for all animals. In addition, it should be possible to use the same criteria for the humane end points.

It is not necessary that all animals undergo all the procedures described in an appendix. The following is permitted: The majority of animals undergo procedures a, b, c, d and e; some of the animals undergo only procedures a, b, c and d; and the remainder of the animals undergo only procedures a, b, c and e. The following is not permitted: Some of the animals undergo procedures a, b, c, some of the animals undergo procedures a, b, c, d and e; and the remainder of the animals undergo procedures a, b, c, g and h.

This means that, for example, generating different animal models through different types of operations generally cannot fall under 1 appendix Description animal procedures. Also all operations that are technically possible within your institution in 1 appendix may seem logical, however, that does not necessarily fall within a project license. Variants of an action with the same objective may fall within one type of animal experiment. Examples include taking blood via different routes and immunizing animals via different routes of administration. Animal experiments involving different animal species can fall under the same type of animal experiment, provided that the actions the animals undergo are similar for each of the animal species. Under these conditions, a pilot experiment in which the required number of animals is determined, a trial in which the most optimal concentration of a substance to be administered is determined, and the final trial may also be described in 1 appendix Description animal procedures. Each of the types of animal experiments listed here should be detailed in a separate appendix Description animal procedures.

Appendix Description animal procedures

Each type of animal procedure listed in Section 3.4.3 should be described in detail. Adhere to the numbers appointed to the individual procedures in Table 3.4.3 (for example 3.4.3.1, 3.4.3.2).

1. General information

1.1, 1.2 Provide general information on the project, including a descriptive and unique title which completely covers the content of the project licence application. This information should be identical to the information provided in the project licence application form.

2. Description of animal procedures

A. Experimental approach and primary outcome parameters

Sub-question 1
Describe the general design of this type of animal procedure with respect to both the primary and secondary outcome parameters and the procedure's objectives. It is not necessary to provide a detailed description of each individual treatment.

Describe the primary and secondary outcome parameters. Justify the choice for these parameters with respect to the purpose of the project. The nature of the primary outcome parameters will be different for each research category. For scientific research, these may include clinical parameters and/or experimental data. For education and training or routine production, these parameters may include learning targets and productivity, respectively.

Sub-question 2
Describe the proposed procedures, including the type, frequency and duration of the treatments. A justification of the selected approach should be provided at G. Keep in mind that specific details relating to the approach must be discussed with the Animal Welfare Body (IvD) before the start of the project. If not all animals undergo the same procedures, indicate under which circumstances a specific treatment is selected.

> Example: Permanent cannulas will be placed in the jugular vein of adult rats (under general anaesthesia), upon which the rats will be housed individually. After a recovery period of at least one week, the animals will receive once or repeatedly (maximum 2 times per day for a maximum of 7 days) a substance containing solution by intravenous injection. Blood samples will subsequently be collected at different time intervals (less than 10 times during 48 hours) using the cannulas to determine the concentration of the substance and the expression of biomarkers.

Sub-question 3
Experimental design: Describe which approach will be used to obtain reliable results. In addition, explain which measures will be taken to ensure that the number of animals will be kept to a minimum in each of the procedures. To justify the maximum number of animals required for each type of animal procedure, both statistical and non-statistical considerations may be included. If it is not possible to use statistical calculations, explain why this is not possible and describe which other considerations were taken into account to determine the number of animals.

In this section, it is not necessary to provide detailed information on power analyses for individual experiments.

B. The animals

Provide for each 'type of animal procedure' information on:

- Species
- Origin of the animals. Please distinguish between
  - animals bred for use in procedures;
  - animals not bred for use in procedures.
  - If animals that are not bred for the use in procedures, are listed in Annex I of the Directive 2010/63/EU, this should also be indicated. If applicable, also indicate whether special categories of animals are used, including non-human primates, endangered species, animals in/from the wild, stray and feral animals.
- Life stage (including, if applicable, foetal forms of mammals in the last third of their developmental period) and the number of animals for each life stage;
- Estimated number of animals (the maximum number of animals you consider to be necessary). The total number of animals requested for the duration of the project should be specified here;
- The gender of the animals. To reduce the killing of 'surplus' animals bred for animal procedures, in general, both male and female animals should be used. If only one gender will be used, justify your choice;
- Genetic alterations;
- The strains, if necessary for achieving the objectives.

  If necessary, additional rows may be added to the table (for example if multiple species are used).

Justify your choices with respect to the objective(s) of this particular type of animal procedure and the principles of replacement, reduction and refinement.

Are special animal categories used? Special animal categories include non-human primates, endangered species, animals in/ from the wild, stray an feral animals and species listed in Annex I of Directive 2010/63/EU. These special categories of animals should, in general, not be used for animal procedures. This may only be allowed if a scientific justification is provided as to why the project's objective cannot be achieved without the use of those animals. For each of these categories, additional requirements have to be met (see below). If applicable, indicate which category applies, provide the requested information and justify your choice.

Special animal categories

- **Apes;**
  - The use of apes if strictly forbidden in the Netherlands.

- **Other non-human primates;**
  - A scientific justification must be provided as to why the project's objective cannot be achieved without the use of this specific type of animal.
  - Other non-human primates may only be used in procedures when the objective of the project relates to:
    a) fundamental research (only if the animals were born in captivity);
    b) preservation of the species;
    c) translational or applied research aimed at avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormalities in humans, animals or plants;
    d) development, manufacture or testing of the quality, efficacy and safety of drugs, food, animal feed and other substances or products.

    If c) and d) are applicable, research must be limited to avoidance, prevention or treatment of debilitating or life-threatening diseases in humans.

- **Endangered species;**

  This concerns species listed in Annex A to Council Regulation (EC) No 338/9.
  - A scientific justification must be provided as to why the project's objective cannot be achieved without the use of this specific type of animal.
  - Endangered species may only be used in animal procedures if the objective relates to:
    a) preservation of the species;

b) translational or applied research aimed at avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormalities in humans, animals or plants;
c) development, manufacture or testing of the quality, efficacy and safety of drugs, food, animal feed and other substances and products.

Note: Animals of endangered species that were born in captivity are listed in Annex B to Council Regulation (EC) No 338/9. The above described restrictions do not apply to these animals.

- **Animals in/from the wild;**
  - Although legal restrictions are applicable, catching wild animals per se is not considered an animal procedure.
  - A scientific justification must be provided as to why the project's objective cannot be achieved without the use of animals in/from the wild.

- **Stray and feral animals;**
  - A scientific justification must be provided as to why the project's objective cannot be achieved without the use of this specific type of animal.
  - A scientific justification must be provided as to why the project's objective cannot be achieved without the use of this specific type of animal.

- **Species listed in Annex I of the Directive 2010/63/EU.**

(species that are not specifically bred for animal procedures).

  - A scientific justification must be provided as to why the project's objective cannot be achieved with the use of animals that are specifically bred for animal procedures.
  - The following species are listed in Annex 1: mouse (Mus musculus), rat (*Rattus norvegicus*), Guinea pig (*Cavia porcellus*), Syrian hamster/ golden hamster (*Mesocricetus auratus*), Mongolian gerbil (*Meriones unguiculatus*), rabbit (*Oryctolagus cuniculus*), dog (*Canis familiaris*), cat (*Felix catus*), frog (*Xenopus laevis, tropicalis; Rana temporaria, pipiens*) or zebra fish (*Danio rerio*).

C. Accommodation and care

The animals should be housed and cared for according to the minimal requirements listed in annex III of Directive 2010/63/EU. The English version can be found at:
Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposesText with EEA relevance

If the minimal requirements cannot be met (i.e. individual housing of social animals, cages with a grid/wire floor and restricted diet), provide justifications for these choices (scientific or animal welfare). Describe the potential adverse effects on the animals and describe what measures will be taken to limit these adverse effects. Any potential additional discomfort caused by alternative housing or care should be discussed at Section F.

D. Pain and compromised animal welfare

Sub-question 1
Indicate whether the animals are likely to suffer from pain. If so, explain which steps during the animal procedures will cause pain and describe how pain will be alleviated. When anaesthesia or analgesia is to be used, specify what measures will be taken to ensure that optimal procedures are used. It is not necessary to provide detailed information on alternative care, substances, dosages and administration methods. These details should be agreed upon with the AWB before implementation. In cases where anaesthesia, analgesia or other pain relieving methods are not to be used, provide scientific justifications for these choices (i.e. when it is not feasible to alleviate pain or when the scientific outcome will become unachievable).

It is forbidden to administer substances that diminish or completely abrogate the animals' capacity to express pain, but do not alleviate pain or reduce consciousness. A thorough scientific motivation is required to obtain authorisation for the administration of such substances.

Sub-questions 2 and 3
Describe the expected adverse effects on the animals' welfare and explain why these effects may emerge. These are not incidental or improbable effects. For example:

- (Alternative) housing;
- Transportation during the procedures;
- Unintended side-effects;
- Genetic alterations (altered phenotype);
- Aging phenomena;
- Experimental procedures (including euthanasia);
- Possible direct and indirect effects of the experimental interventions or treatments.

Sub-question 4
Describe which measures will be taken to prevent or minimise these unexpected effects. Information regarding adaptations to housing or specific postoperative care may also be provided here.

E. Humane endpoints

Sub-question 1
A procedure may be prematurely ended for an individual animal (implementation of the humane endpoints) if a) the level of distress of this individual animal exceeds the project's upper limit; b) the procedure's scientific endpoint has been achieved (i.e. a pre-described tumour size); or c) the scientific endpoint can no longer be achieved (i.e. the side-effects in the animal or its response to the procedures can disturb further treatment or observations).

Indicate whether, during this animal procedure, circumstances may arise which would require the implementation of humane endpoints to prevent further distress. If so, describe the criteria that will be used to identify the humane endpoints (i.e. a certain weight loss, changes in behaviour or body posture, appearance/disappearance of certain biomarkers). Describe why these criteria have been selected. In addition, describe how the animal will subsequently be treated.

In most cases, the animal will be killed, while in specific cases, it may be possible to prematurely end the procedure for an individual animal by alleviating or ending the distress without killing the animal. If a pilot study will be performed to determine the humane endpoints, please describe this in Section 3.4.1.

If death will be the outcome of the animal procedure, specify why humane endpoints cannot be applied and which measures will be taken to minimise the adverse effects of this procedure. For more information on humane endpoints, see https://www.humane-endpoints.info/en/what-are-humane-eindpoints

Sub-question 2
For each species, indicate the percentage of animals expected to have a chance of meeting the criteria for humane endpoints.

F. Classification of severity of procedures

Provide information on the experimental factors (e.g. treatments, housing, genotype, transport) contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). The classification criteria are listed in Annex VIII of the Directive 2010/63/EU. The English text of this directive can be found at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&qid=1415627717806&from=EN

'Terminal' means procedures performed under general anesthesia at the end of which the animal does not regain consciousness. The killing of animals without performing a prior procedure (under anesthesia) on this animal (such as re-killing to obtain tissue and organs) is classified as mild. In

the Netherlands, a licence is required to kill animals for the sole purpose of harvesting tissue and organs.

If some of the animals/animal groups are likely to experience a different level of reduction in welfare, each classification should carry with it information regarding the percentage of the animals that will be affected. You can think of donor animals versus recipient animals, animals that do / do not experience a serious infection and animals that do / do not undergo surgery. Finally, indicate the cumulative distress for each animal species as a percentage of the total number of animals in this appendix. You may also display this information in a table.

If procedures are classified as 'severe', justify why this cannot be avoided. Animal procedures that can potentially lead to prolonged and severe pain, suffering or anxiety, which cannot be alleviated, can only be permitted as an exemption.

G. Replacement, reduction and refinement

Sub-question 1
Justify the chosen experimental design as described in Section A with respect to the principles of replacement, reduction and refinement (3Rs). Describe which methods for replacement, reduction and refinement have been/will be applied in the experimental design. Describe each of the 3Rs separately.

Replacement: Explain why the objectives of this project cannot be achieved without the use of animals. Describe which other options have been taken into consideration and explain why these options were not considered applicable for this project. You can think of in vitro techniques, research in humans and use of, for example, offal.

If your applications focuses on animal husbandry, you should also explain to which extent alternatives outside the context of the project, such as alteration of current livestock farming systems, can be used to achieve the objectives. This may, for example, be applicable for research focused on animal diseases that are caused by current livestock farming systems. Adjusting housing conditions may be sufficient to solve the problem without having to perform animal procedures.

Reduction: Both the number of animals and the justification of this number are important factors in the project evaluation. Although too many animals are not acceptable from an ethical perspective, too few animals are also not acceptable when the number is too low to achieve the project's objectives. Describe which measures will be taken to minimise the number of animals. Information may be provided on, for example, the performance of systematic reviews, the use of *in silico* models, the use of *in vitro* experiments, phasing in the project, go/ no go moments, combining tests, and the use of imaging techniques.

If a systematic review has been carried out, *in silico* models have been used or *in vitro* experiments have been performed, these should be described here. Results of a systematic review can be described at question 3.1. of the project proposal.

Refinement: Here justification should be provided regarding the choice of the above described species. In addition, information should be provided explaining why the proposed procedures are the most refined for the intended purposes. The term 'most refined procedures' refers to experimental procedures that will yield reliable results with minimised animal suffering and a minimal number of animals.

Furthermore, describe which measures will be taken to minimise the decline in animal welfare. Information may be provided on, for example, observation strategies, pain relieving methods, the envisaged humane endpoints when animals suffer more than anticipated, training of animals, optimising techniques, technological developments such as imaging methods, ex vivo experiments on cells of untreated animals, and inducible animal models.

If the project is (also) aimed at the development of animal free models and/ or more refined techniques, and the knowledge obtained in this project may be applied during the course of the current project, describe how this knowledge will be implemented.

Sub-question 2
Indicate whether adverse environmental effects can be expected. If substantial negative environmental effects (in nature and/or scale) may be expected, describe which measures will be taken to minimise these effects.

H. Re-use

Re-use refers to an animal procedure during which an animal that has already been used in at least one prior animal procedure, is used when a different animal that has not previously been subjected to an animal procedure could be used instead. If data obtained during previous procedures carried out on an animal is essential for the subsequent procedure, these procedures are not considered to constitute re-use. Similarly, instrumentation of an animal (i.e. telemetry) or a previous surgery (i.e. gonadectomy) may render an animal especially suitable for subsequent procedures. These procedures are also not considered re-use procedures.

Re-use is allowed when:

- the actual level of distress in the previous procedure was 'mild' or 'moderate'; and
- the animal's general state of health and well-being has been fully restored after the previous procedure; and
- the proposed procedures are classified as 'mild', 'moderate' or 'non-recovery'; and
- the proposed procedures are in accordance with veterinary advice. Deviation from this advice should be motivated.

If applicable, justify why re-use of the animals should be considered acceptable.

The cumulative discomfort during prior and current procedures should not be taken into account while assessing the severity classifications.

The decision to reuse an animal at the end of an animal experiment is made by a veterinarian or other competent person. An animal is killed when it is likely to suffer moderate or severe pain, suffering, distress or will be left with permanent harm as a result of the tests.

I. Repetition

This question refers to legally required research. Describe what measures will be taken to ensure that the proposed animal procedures have not already been performed. If at least one of the proposed procedures has already been performed, justify the necessity of repetition. Please note that each member state has to acknowledge procedures performed in another member state that were performed in accordance with European legislation.

J. Location where the animal procedures are performed

If the animal procedures will not take place in a licenced establishment, explain why this is necessary and describe how the requirements for animal welfare, housing, care and treatment will be satisfied. Animal procedures may, for example, be performed in the wild, at a zoo, or at a livestock farm that does not belong to a licenced establishment.

If animal procedures are to be carried out at a new location, this location must be registered with the NVWA before animals are housed and / or animal procedures are carried out at this location. An NVWA inspector will then inspect the location. Indicate here whether the location is registered with the NVWA and how it is ensured that the animals are adequately accommodated and cared for. Adequate housing and care means at least in accordance with the requirements set out in Annex III of Directive 2010/63 / EU or comparable.

K. Method of killing

Sub-question 1
Indicate whether animals are killed during or after the animal procedures. If animals are killed, Justify why it is necessary to kill animals during or after the animal procedures. If animals are not killed, describe the destination of the animals.

Sub-question 2
Animals may only be killed using methods listed in Annex IV of Directive 2010/63/EU. For the English version of Directive 2010/63/EU see: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&qid=1415627717806&from=EN

The CCD may only issue a licence for animal procedures that require the use of alternative methods of killing:

- if the scientific justification convincingly argues why the objective cannot be achieved using an approved method;
- if the proposed method is as humane as the approved methods and exemption is granted by the Minister.

If a method is used that is listed in Annex IV of Directive 2010/63/EU that may only be used if other methods are not possible, provide justification for your choice.

For a few methods described in appendix IV, additional conditions apply. For example, rodents may only be killed by decapitation, if other methods mentioned in Annex IV cannot be used. If such a method is used, describe the method and provide justification for using this particular method.

Any potential additional discomfort caused by such an alternative method of killing should be included in the assessment of the severity classifications in Section F.

Sub-question 3
In many cases, scientific or welfare reasons make it necessary to kill the animal. If the animal is still alive at the end of the procedure, there are inherent ethical dilemmas that must be addressed. When considering keeping an animal alive after completing the procedure, you should consider the legal requirements for animal welfare management, particularly those set forth in Articles 17 and 19 of Directive 2010/63/EU.

If animals are killed for non-scientific reasons, justify why it is not feasible to release or rehome the animals. In considering rehousing or release, Article 19 of Directive 201/63/EU applies as does the Animal Welfare Body's opinion on the rehousing scheme (Article 14c(1)(e) Wod).

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