Centrale Commissie Dierproeven

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:

a. submit an **application for a project licence**;
b. 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 alter the scope of the project licence;
c. **notify** the Central Authority for Scientific Procedures on Animals (CCD) of minor alterations to a project. It is sufficient to notify the CCD when the proposed alterations do not adversely affect the welfare of the animals.

For more information on amendments and notifications, see the official policy rules 'Meldingen in het kader van een projectvergunning' (Staatscourant 2017, 8037) and the practical guidance document on the website of the CCD.

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 by/in licenced user establishments. For this, the competent authority is the 'Netherlands Food and Consumer Product Safety Authority', also known as the 'NVWA'. The institutional license number (*'deelnemernummer NVWA'*) should be provided here. 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 licence.

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/principal investigator)

This person is responsible for writing the project licence application and performing the procedures according to the project licence. For this, this person should meet the requirements defined 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.

1.5 Acting responsible researcher

A second responsible researcher can be appointed, and he/she must also meet the requirements defined 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 qualified person.

1.7 Details Animal Welfare Body

Contact details of the institutional 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 scope 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.
For an amendment, 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 parameters' 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 parameters' and F 'Classification of severity of procedure', but also D 'Pain and compromised animal welfare', E 'Humane endpoints' and G 'Replacement, reduction, refinement'. You may 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 of 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 amendment. The altered NTS will not be published until the CCD has authorised the amendments.

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 portfolio 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 content of the original NTS is no longer reflecting the project, an updated NTS can be provided. This may, for example, occur when fewer animals are used than originally requested or parts of the project will not be carried out.
The altered NTS will be published on the website of the CCD.
For more information of notifications, see the official policy rules 'Meldingen in het kader van een projectvergunning and the corresponding guidelines.

3. About you

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 not exceeding 5 years.

3.2 Title project
Use a descriptive and unique title which covers the content of the project application.

3.3 Title non-technical summary
The title of the non-technical summary should be provided here. This title may be different from the title provided in 3.2 (e.g. must be in Dutch language). This title should be informative, and 'non-technical'.

3.4 Animal experiments committee (DEC)
Provide here the name and contact details of the preferred animal experiments committee (optional).

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 the project licence application needs to be comprehensive (not require any additional documents). Before submitting the application, ensure that all necessary appendices have been enclosed.

6. Ondertekening

6.1 Signature
The licence application form must be 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 declares 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 NTS should be written in Dutch. To write the NTS, you must use the form provided by the European Commission. The questions and explanatory text in this format (from version 1.1.) will automatically be displayed in Dutch upon indicating that the NTS is written in Dutch

The responsible researcher is responsible for writing the NTS. The Animal Welfare Body (IvD) should advise the applicant during this process to optimise the content and accuracy. The project should be explained clearly by using non-technical phrasing that can be understood by the general public. Avoid the use of concealed language, technical details, abbreviations and jargon. Ensure that the text is relevant, unambiguous and comprehensible. The NTS should be anonymous (both regarding establishment and persons) and should not contain intellectual property or confidential information.

The NTS will not be published until the project licence application has been granted by the CCD. The NTS should be submitted in the excel format provided and ready for publication.

See the 'NTS guidance document for users' on the website of the CCD for more information on writing the NTS

Project proposal

The project proposal is the core document of the project licence application. It must include all information that is necessary for the legally required ethical evaluation and project authorisation. The purpose of the project proposal is to inform the DEC and the CCD of the objectives of the project 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 for the design of the project.
It is therefore essential to highlight the relevance of the project and how it is structured. In addition, the project's proposal should explain how the project complies with the legislation in force. Please note that experts from multiple disciplines will be involved in the evaluation of your project.

Readability
The project licence application must be comprehensive and well-structured without any redundant or irrelevant information. The types of animal procedures are to be described in the appendix forms. Other types of appendices (not compulsory) should only be enclosed with the project licence application when they are essential to the evaluation and authorisation of the project. In that case, each of these appendices should be cited in the text. Moreover, the relevance/importance of the appendices should be indicated (how they support, or justify, or illustrate the application in a useful way).

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 animal 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 explained 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. This title should be identical to the title provided in the project licence application form.

2. Categories

The project should be designed to serve defined purposes. At least one broad category of project purposes must be selected here. These purposes should be consistent with those selected in the non-technical summary. More than one category may apply. For more information on the definitions of the purposes, see implementing decision EU/2020/569 (article 10.1).
Please note that breeding of genetically altered animals is defined as an animal procedure when the welfare of the animals is affected by the modifications (an intended and exhibited harmful phenotype) or requires invasive procedures for genotyping (if not aligned with identification, which is not a regulated scientific

procedure). For more information on breeding GA animals see the document 'Herziene Wet op de dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren'**.**

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. If a systematic review has been performed, the results can be described here.
What type of contextual or background information will be instrumental for evaluation of the project depends on the selected category/categories.

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.
- 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.

For Protection of the natural environment in the interests of the health or welfare of human beings or animals:

- Explain how the scientific use of animals relates to the protection of the natural environment in the interests of the health or welfare of human beings or animals.

Preservation of species:

- Describe the current situation regarding the species to be preserved (the species used in procedures and/or others) and how this is addressed now.
- Describe how the project will promote the preservation of species.

For regulatory use or routine production:

- Explain for which regulatory requirements (proposed use and/or market authorisation) the proposed research is considered necessary.
- Indicate the competent authorities that will use the outcomes for evaluation of, e.g., efficacy or safety..
- Explain which regulatory requirements will be satisfied by these procedures (see article 10.3.1. of the Implementing decision EU/2020/569 for a detailed breakdown of types of regulatory requirements).

For routine production:

- Describe the product type, how this project employs established methods and what are the typical uses of the product(s).
- In case the products will be supplied to third parties, indicate the expected demand for this/these product/products.

For higher education or training:

- Regarding higher education: explain how the use of animals fits in the educational program (e.g., curriculum, elective course, or postgraduate course) and provide information on the defined learning outcomes.
- For the acquisition, maintenance or improvement of vocational skills: explain the types of professionals needing to acquire, maintain or improve vocational skills, what vocational skills and how the use of animals contributes. Also indicate any formal requirements for this training (e.g., the acquisition or maintenance of professional certificates).

For projects relevant for livestock farming

- 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
- New developments leading to optimisation of the 3Rs can be mentioned in section G of the appendix Description of animal procedures.

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 use this summary for the purpose of a formal Retrospective Assessment. You therefore do not need to include information on the use of animals. New developments leading to optimisation of the 3Rs can be mentioned in section G of the appendix Description of animal procedures.

3.2. Purpose

3.2.1. Immediate goal and ultimate goal

Describe both the direct objectives and the ultimate purpose or goal (beyond the project). Explain what you are aiming to achieve, confirm, investigate, produce, test or obtain by undertaking this project. While the direct objectives 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. How the subobjectives and project components relate has to be described in Section 3.4 (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 and the infrastructure. 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. 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', 'Wet Natuurbescherming', 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 other legislation states that animals included in animal procedures may be exempted from that particular legislation, such as the 'Transport verordening', the CCD must assess whether the specified conditions are met.

Animal welfare may also be reduced as a result of compliance to other laws and regulations. For example, the need for 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 in case the regulatory requirements in Europe have implemented 3Rs alternatives to the effect that fewer (or no) animals are involved, or milder procedures.
If, in comparison to European regulations, more animals are required to meet product registration requirements, 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 objectives. 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.

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, other domestic animals or wild fauna, 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, please indicate here how this may impact the field.
For applications relevant for livestock farming, 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. Project 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 different types of 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 schematically, e.g. using a time line. Indicate any interdependence between de different components of the project including any go/ no go moments for a next step.. Describe, for each of the go/ no go moments, the criteria for deciding whether or how the project will be continued.
The project proposal should highlight the components involving the use of animals. A referral to project components not requiring the use of animals may be made in case they are essential to the realisation of the main objectives. For each component, the 'type of animal procedures' that will be performed should be outlined.

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 how this strategy affects the use of animals (in terms of numbers and/ or animal welfare). Information about the types of animal procedures should be provided in the corresponding appendices 'Type of animal procedures'.

3.4.3. Type of animal procedures
The term 'type of animal procedure' refers to a specific document (Appendix Description animal procedures) detailing the use of animals (animal uses are not to be detailed here in the 'strategy' section). Here, those annexes are to be listed by number and short title. Each animal in the project should be assigned to a specific appendix. In the exceptional case that the use of an animal may start in one appendix and the animal is then transferred to another one, this should be mentioned in the appendices concerned.
One appendix Description animal procedures should detail similar animal uses in a comprehensive way. 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.

However, 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 allowed: 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.

Please note that it is allowed to include small variations of a procedure in a single appendix, such as administration of cells or compounds through different routes. Under these conditions, a pilot experiment in which the required number of animals is determined, a test in which the most optimal concentration of a substance to be administered and the final test can also be described in 1 appendix.

Appendix Description animal procedures

Each type of animal procedure listed in Section 3.4.3 should be described in an Appendix.

1. General description
1.1. and 1.2. information on the establishment
1.3. This information should be identical to the information provided in item 3.4.3 of the project licence application form, the serial number formatted as 3.4.3.1, 3.4.3.2, et cetera, and the title of the appendix as listed in item 3.4.3 of the project proposal.

2. Description of animal procedures
A. Experimental approach and primary outcome parameters

Sub-question 1

Describe the general design of this type of animal procedures 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 different types of projects. For scientific research, these typically include experimental data and specimens. For education and training or routine production, these parameters will address learning targets and product supply, respectively.

Sub-question 2

Describe the proposed procedures, including the type, frequency and duration of the techniques/treatments. A justification of the selected approach should be provided at G. If not all animals will undergo the same procedures, indicate under which circumstances a specific treatment is selected. Please describe this comprehensively, in a way that sufficient insight is provided regarding what may happen to a single animal in this appendix.

Sub-question 3

Design of the experimental procedures: 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 estimates for statistical power, explain why this is not possible or feasible 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 experimental designs.

B. The animals

Sub-question 1

A table is to be completed to provide information on the animals. If more than one animal species is used, new rows can be included in the table. Provide this information taking into account the definitions applied for annual statistical reporting of animal uses (detailed and defined in Implementing Decision (EU) 2020/569),

- Species. Please specifically adhere to the species nomenclature in the

drop-down menus in the tab 'expected harms' in the NTS format; in addition, a common name can be provided.

- Origin of the animals. Please distinguish between 1) animals bred for use in procedures, and 2) animals not bred for use in procedures. If species listed in Annex I of the Directive 2010/63/ are not purpose bred for the use in procedures, this should also be indicated. Also indicate whether special categories of animals are used, including non-human primates, endangered species, animals in/from the wild, or stray/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 avoid the creation of 'breeding surplus' (animals purpose bred for animal procedures, but not used), in general, both male and female animals should be used. If only one gender will be used, justify your choice.
- Genetic alterations
- The strain(s), only to be specified in case of necessity for achieving the objectives.

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

Sub-question 2

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

Are special animal categories used? Special animal categories include non-human primates, endangered species, animals in/ from the wild, stray/feral animals and deviations from the requirement that species listed in Annex I of Directive 2010/63/EU should be purpose bred (obtained from licensed breeder/supplier of purpose bred animals). 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
○ **<u>Mensapen;</u>** ○ The use of apes is strictly forbidden in the Netherlands. ○ **<u>Other non-human primates;</u>** ○ 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. o Other non-human primates may only be used in procedures when the objective of the project relates to: ○ fundamental research (only if the animals were born in captivity); ○ preservation of the species; ○ translational or applied research aimed at avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormalities in humans, animals or plants; ○ 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, with the exception of non-human primates, may only be used in animal procedures if the objective relates to:

- preservation of the species;
- translational or applied research aimed at avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormalities in humans, animals or plants;
- 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/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**;

(animals of species that should be purpose bred (licensed breeder/supplier) but are acquired from other sources).

  - 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*), some frog species (*Xenopus laevis, X. tropicalis; Rana temporaria,R. pipiens*) and zebra fish (*Danio rerio*).

C. Accomodation 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:
http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&qid=1415627717806&from=EN
If the minimal requirements cannot be met (i.e. individual housing of social animals, cages with a grid/wire floor), 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 addressed in 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 with the IvD in preparation of implementation.
In cases where pain can occur but 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 be compromised).
It is forbidden to apply pharmacological regimens that diminish or completely abrogate the animals' capacity to express pain, but insufficiently alleviate pain or reduce consciousness. A thorough scientific justification is required for lifting this ban.

Sub-questions 2 and 3

Describe the expected adverse effects on the animals' welfare and explain why these effects may emerge. For example:

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

It is not necessary to describe incidental or improbable welfare complications.

Sub-question 4

Describe which measures will be taken to prevent or minimise these adverse effects on animal wellbeing. Information regarding, e.g., 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- defined 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 and how these relate to the scientific endpoint. 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 scientific end point of the animal procedure, justify 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 http://www.humane-endpoints.info/eng/

Sub-question 2

For each animal model, indicate the likely incidence.

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 combined 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

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 numbers of the animals that will be classified accordingly. 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.

The term 'non-recovery' refers to procedures performed using general anaesthesia from which animals will not recover. Instead, the animals will be killed humanely.

In the Netherlands, a licence is required to kill animals for the sole purpose of harvesting tissue and organs for scientific purposes. For the purpose of national statistical reporting, the severity of this procedure should be classified as 'mild'.

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 by exemption by the Minister.

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 feasible to achieve the objectives this project. You can think of in vitro techniques, research in humans, use of animals below the welfare impact threshold defining regulated animal procedures for scientific purposes, and use of animal corpses and waste products from slaughterhouses.

If your application has livestock farming as the focus, 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 the need for 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 approach 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, statistical methods, combining test groups (e.g., sharing control groups between experiments), and the use of longitudinal scientific readouts, e.g. 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/models. 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 welfare impacts. 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 otherwise 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 development 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. Please refer to Implementing Decision (EU) 2020/569 for further definition and interpretation, and how reuse differs from continued use.
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 only allowed if:

- the actual level of severity 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.
-

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.

At the end of a procedure, a decision to keep an animal alive shall be taken by a veterinarian or by another competent person. An animal shall be killed when it is likely to remain in moderate or severe pain, suffering, distress or lasting harm.

I. Repetition
This question refers to regulatory testing. 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

Animal procedures are to be situated in a licenced user establishment. A Dutch project licence is only valid on national territory.
This question does not refer to a situation where the animal procedures, in the context of a collaboration, are conducted in another licenced establishment within The Netherlands. It also does not refer to the housing of animals before or after the procedures.
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 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, and such approaches need to be brought to the attention of the EC.

If a method is used that is listed in Annex IV of Directive 2010/63/EU, but 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 in case no prior sedation or general anaesthesia is applied.
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 it is necessary to kill the animal for scientific or welfare reasons. In case the animal will be alive at the end of the procedure, there are inherent ethical dilemmas to be addressed.
When considering to keep an animal alive after completing the procedure, please take into account the legal requirements to manage animal welfare, specifically those laid down 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. When considering rehoming or release, article 19 of directive 2010/63/EU applies as well as the advice of the Animal Welfare Body on the rehoming scheme (Article 14c, sub 1e of the Animal Procedures Act).

Disclaimer: The project licence application form and the guidelines to this licence application form are translated from the corresponding Dutch documents. These English translations have been generated for information purposes only. In case of discrepancies between the original Dutch documents and the translated English versions, the original Dutch versions always prevail.