

**National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes**

**Working document on Non-technical Project Summaries**

Brussels, XX-XX XXXX 2021,  
replacing consensus document of 23-24 January 2013

The National Contact Points of the Member States responsible for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes ('the Directive') and the Commission agreed to discuss the practical implementation of the requirement under Article 43 of the Directive with a view to finding a common approach throughout the EU.

Regulation (EU) 2019/1010 of the European Parliament and of the Council of 5 June 2019, amending Article 43 of the Directive, and the related Commission Implementing Decision 2020/569/EU set out legally binding common formats and data content for non-technical project summaries and the results of retrospective (project) assessments. The consensus on the approach discussed and endorsed at the meeting of 23-24 January 2013, and the revised legal context, were used as the basis to update and further develop this guidance. The outcome is presented below to promote uniform implementation and application of the Directive.

**Disclaimer:**

**The following is intended as guidance to assist the Member States and others affected by this Directive to arrive at a common understanding of the provisions contained in the Directive. All comments should be considered within the context of Directive 2010/63/EU on the protection of animals used for scientific purposes, as amended by Regulation (EU) 2019/1010 of the European Parliament and of the Council of 5 June 2019.**

**Only the Court of Justice of the European Union is entitled to interpret EU law with legally binding authority.**

**The table of contents:**

**Table of Contents**

Introduction..... 3

Legal background..... 3

    Non-technical project summaries and results of retrospective assessment ..... 3

    Detailed content for the non-technical project summaries..... 4

    Publication of non-technical project summaries and updates thereof..... 4

Benefits of non-technical project summaries ..... 5

General guidance for the drafting of non-technical project summaries..... 5

    How to ensure that non-technical project summaries are accurate and representative of the project ..... 6

    Ensuring the safeguarding of intellectual property and confidential information ..... 6

Specific guidance on the content to be included in the NTS template ..... 6

    Title of the project..... 6

    Duration of project (in months)..... 6

    Keywords ..... 7

    Purpose of project..... 8

    Objectives and predicted benefits of the project..... 8

    Predicted harms..... 10

    Fate of animals kept alive..... 13

    Reasons for the planned fate of the animals after the procedure..... 13

    Application of the Three Rs..... 14

        1. Replacement..... 14

        2. Reduction..... 15

        3. Refinement ..... 16

    Project selected for retrospective assessment..... 17

Appendix I – Template for the non-technical project summary ..... 18

Appendix II – Illustrative examples of completed non-technical project summaries..... 19

## Introduction

One of the key aims of the Directive is to enhance transparency and to ensure that the public is objectively informed about the use of animals for scientific purposes. This is clearly expressed in both Recital 41 and Article 43 of the Directive. The main tool that is used for this purpose is the publication of non-technical project summaries (NTS) and the results of retrospective assessments (RA) of approved projects authorisations.

In 2013, the European Commission issued a working document with the aim of harmonising the approach to the completion of NTS within the European Union. NTS have proven to be a useful tool in promoting transparency, contributing to the sharing of good practices in relation to the Three Rs, and helping to avoid duplication of animal testing. It was recognised however that in order to further improve transparency and to establish an open access EU database on the use of animals for scientific purposes, some amendments to the reporting obligations were necessary. The legal basis for these amendments is Regulation (EU) 2019/1010 on the alignment of reporting obligations and Commission Implementing Decision 2020/569/EU (replacing Commission Implementing Decision 2012/707/EU).

The aim of the legislative changes is the creation of a central, open access, searchable database for NTS and related RA. Commission Implementing Decision 2020/569/EU (hereafter 'the Decision') sets out a common format for submitting this information to the Commission. To achieve the objective of enhancing transparency, and realise the maximum benefits of publishing NTS, harmonisation in the reporting of NTS and RA across Member States is essential.

Thus, the aim of this document is to provide guidance for end-users on how to complete NTS using the new template defined in Annex I Part A of the Decision and included in Appendix I of this document. Illustrative examples of completed NTS are provided in Appendix II. The examples are grouped in four categories (related to the purpose of the project in each case):

1. Basic research
2. Translational and applied research
3. Regulatory testing
4. Education and Training

Where appropriate, the specific guidance on the content will include text excerpts from the illustrative examples captured in Appendix II.

## Legal background

### Non-technical project summaries and results of retrospective assessment

#### **Article 43**

*1. Subject to safeguarding intellectual property and confidential information, the non-technical project summary shall provide the following:*

- (a) information on the objectives of the project, including the predicted harm and benefits and the number and types of animals to be used;*
- (b) a demonstration of compliance with the requirement of replacement, reduction and refinement.*

*The non-technical project summary shall be anonymous and shall not contain the names and addresses of the user and its personnel.*

*2. Member States may require the non-technical project summary to specify whether a project is to undergo a retrospective assessment and, if so, set out the deadline. In such a case, from 1 January 2021, Member States shall ensure that the non-technical project summary is updated within six months of the completion of the retrospective assessment with the results thereof.*

*3. Member States shall, until 31 December 2020, publish the non-technical project summaries of authorised projects and any updates thereto. From 1 January 2021, Member States shall submit for publication the non-technical project summaries, at the latest within six months of authorisation, and any updates thereto, by electronic transfer to the Commission.*

*4. The Commission shall, by means of implementing acts, establish a common format for submitting the information referred to in paragraphs 1 and 2 of this Article. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 56(3). The Commission services shall establish and maintain a searchable, open access database on non-technical project summaries and any updates thereto.*

### **Article 39**

*1. Member States shall ensure that when determined in accordance with Article 38(2)(f), the retrospective assessment shall be carried out by the competent authority which shall, on the basis of the necessary documentation submitted by the user, evaluate the following:*

- (a) whether the objectives of the project were achieved;*
- (b) the harm inflicted on animals, including the numbers and species of animals used, and the severity of the procedures; and*
- (c) any elements that may contribute to the further implementation of the requirement of replacement, reduction and refinement.*

*2. All projects using non-human primates and projects involving procedures classified as 'severe', including those referred to in Article 15(2), shall undergo a retrospective assessment.*

*3. Without prejudice to paragraph 2 and by way of derogation from Article 38(2)(f), Member States may exempt projects involving only procedures classified as 'mild' or 'non-recovery' from the requirement for a retrospective assessment.*

### **Detailed content for the non-technical project summaries**

Article 43 of the [Directive](#) provides the key components that have to be included in the NTS. These have been broken down into sub-components which are detailed in Annex I of the [Decision](#). The template to be used for completion of NTS may be found in Appendix I.

However, additional guidance is considered helpful to aid project applicants to draft meaningful, clear and succinct NTS with a view to promoting consistency within and between Member States.

### **Publication of non-technical project summaries and updates thereof**

As outlined in Article 43 of the [Directive](#), Member States are required to ensure that the NTS is published within six months of the authorisation of the project. The publication is done using an electronic transfer to the European Commission that will host an open-access, searchable EU database.

It is optional whether or not the Member State decides to update NTS following an RA (Article 43(2)). Only those Member States that have (in their national legislation transposing the [Directive](#)) stipulated that NTS shall specify whether a project is to undergo a RA, will be required to update the NTS with the results of the RA. In such cases, the NTS must be updated within six months of the completion of the RA by the relevant competent authority.

NTS and the results of RA can be prepared using any of the Union languages. The EU database will hold the NTS and the results of RA in their original language. However, searches can be undertaken using any of the Union languages. To facilitate this, certain terms from the templates will be available for searches in all Union languages. In addition, an increasing number of intuitive keywords are expected to evolve over time on the basis of searches carried out. This will allow datamining of the entire database, irrespective of the language in which the NTS, or their updates, were submitted. It is for the user of the database then to translate the search results as necessary.

### **Benefits of non-technical project summaries**

A well-written NTS can:

- Enhance openness and transparency around the use of animals in research.
- Facilitate improved accessibility and understanding of different animal use areas amongst the public and non-governmental organisations (NGOs).
- Encourage scientists to develop and improve their communication skills and better explain their research interests to the public.
- Improve the quality of the scientific information available to the public and avoid the spread of misinformation.
- Support the sharing of good practices in relation to the 3Rs.
- Support evidence-based policy making by competent authorities.

### **General guidance for the drafting of non-technical project summaries<sup>1</sup>**

#### **Content of non-technical project summaries**

- Project applicants should be mindful that potential readers of the NTS will be persons unfamiliar with scientific work.
- Only language and terminology which will be easily understood by the public should be used in the NTS. For example, 'under the skin' rather than 'subcutaneous, or 'high blood pressure' rather than 'hypertension' and if necessary, any complex terms should be adequately explained.
- Input from a 'lay person' in the project application process should encourage the development of easily understood NTS.
- The potential benefits of the project proposal should be clearly stated and realistically described. Broad, high level or overstated claims in relation to what the project has the potential to achieve should be avoided.

---

<sup>1</sup> The German Centre for the Protection of Laboratory Animals (Bf3R) has developed a 6-episode video guidance on drafting NTS. It summarises the benefits of publishing NTS and instructs on writing comprehensibly for a lay readership. The videos with English subtitles can be viewed here:

<https://www.youtube.com/playlist?list=PLn53ZjMqXoP5UWmoOGosNa4MCLiSLvSU0>

- It is important that any potential benefits that may be accrued from the work, as well as potential welfare costs to animals, are described in a manner consistent with the information included within the project application.

### **How to ensure that non-technical project summaries are accurate and representative of the project**

- The local Animal Welfare Body may be helpful in assisting on content and accuracy.
- As part of the project evaluation process, the competent authority should ensure that the NTS is accurate and representative of the project. The project should not be authorised until a satisfactory NTS is completed.
- The National Committees for the protection of animals used for scientific purposes may be helpful in reviewing the consistency of NTS submissions retrospectively.
- Research area-specific umbrella organisations can play a role in providing guidance on how to express subject-specific terminology and type of work in a language that is accessible to the general public.
- Public confidence in the benefits of publishing NTS may be eroded if the information included within them is inaccurate, incomplete or uninformative.

### **Ensuring the safeguarding of intellectual property and confidential information**

Article 43 requires that intellectual property (IP) and confidential information shall be safeguarded. The NTS shall be anonymous and shall not contain the names and addresses of the user and its personnel. The NTS shall not violate proprietary rights or expose confidential information (Article 43(1)). Applicants for projects should be aware of these constraints, know that NTS will be made publicly available, and that it is their responsibility to ensure that the NTS included in the application does not contain such information.

|   |
|---|
| <h3><b>Specific guidance on the content to be included in the NTS template</b></h3> |
|---|

#### **Title of the project**

*Maximum length 500 characters*

Ideally, the title should contain all the elements to distinguish the project from others, and should provide conclusive information for an expert readership whilst providing a general sense of the objective of the project for a non-specialist reader.

The title shall be the same as the title included within the project application. When considering the title of the project, it is important to be aware that it must describe the project as precisely as possible and any abbreviation, unless is widely accepted, should be written in full to avoid confusion.

#### **Duration of project** (in months)

Please enter the anticipated duration of the research project. This should be a whole number: 1-60 months.

Typically, this number will be the duration requested in the project application. Note that competent authorities for project evaluation/project authorisation may amend the requested duration prior to project approval. In all instances however, this number should equate to the total duration of the authorised project. In example 4 in Appendix II, '90 months' has been entered for the project duration.

However, as per Article 40 (3), project authorisations shall be granted for a period not exceeding 5 years. Therefore, the maximum project duration permissible is 60 months.

## **Keywords**

*Maximum of 5 keyword entries. Each entry may contain white spaces, but must not exceed 50 characters.*

Keywords are an entry point for members of the public searching for NTS in the database. They may also be used by scientists searching for projects related to particular areas of research. The keyword section of an NTS should summarise the project and facilitate searches by lay members of the public, whilst simultaneously enabling searches by scientists in order to identify projects in specific scientific areas of interest. Therefore, both more general and specific key words should be used.

In general, between three and five keywords entries is appropriate, with five the maximum number permissible. A keyword does not have to consist of a single word – it can also be a phrase or a term, e.g. the term ‘mesenchymal stem cell’ would be classed as a single keyword. Keywords should not be repeated if they are already captured within the *Title of the project* field or in other parts of the NTS (e.g. in the *Species* or *Purposes* field). For example, in illustrative example 6 (Appendix II), *rabbits* and *mice* have been included as keywords. As these species are captured in the *Species* field within the *Expected harms* tab, it is not necessary to include them as keywords and they could be replaced by words/phrases/terms that are more informative about the specific project (e.g. *orthopaedic surgery*, *medical devices*). Additional examples of both good and poor quality keyword selections can be found in Appendix II.

The **project purpose** (see section below) is selected from a dropdown menu. The available options capture both the primary project purpose (e.g. Basic research), and the appropriate sub-field (secondary purpose), where relevant (e.g. Basic research - oncology). In some cases, particularly under the primary project purpose ‘Regulatory use and routine production’, further information may be required in order to accurately describe the end-use of the animal. In such cases, the keywords chosen should include a relevant third level project purpose.

This is particularly relevant for the grouped category ‘Regulatory use and routine production - routine production by type’ where the end-use (third level project purpose) is antibody production. In such instances, *antibody production* should be included as a keyword. Furthermore, in some instances antibody production may be performed within the framework of basic/translational and applied research projects. Where this is the case then *antibody production* should be included as a keyword.

Inclusion of more specific end-uses as keywords may also be beneficial for other grouped categories. For example, projects approved under the primary purpose ‘Regulatory use and routine production’ may represent large scale or generic project authorisations, with testing being performed for a wide variety of third level purposes. As the dropdown menu can only facilitate the selection of a primary and secondary level project purpose, in instances such as these, the third level project purpose should be captured in the *keywords* field, to give a clear indication of the reason it is necessary to perform the testing e.g. :

- for a project with the project purpose ‘Regulatory use and routine production’ (primary level) - *quality control* (including batch safety and potency testing)’ (secondary level), sample keywords could be *pyrogenicity testing* or *batch potency testing*.
- for a project with the project purpose ‘Regulatory use and routine production’ (primary level) - *toxicity and other safety testing including pharmacology’* (secondary level) sample keywords could be *acute ecotoxicity*, *genotoxicity* or *pharmacokinetics*.

It is also required to include *creation of genetically altered animals* as a keyword where relevant.

On a technical note, applicants should be aware that NTS submissions to the centralised database will fail the validation process unless at least one keyword is entered.

### **Purpose of project**

It is possible to assign more than one project purpose to a given project proposal. However, the more precisely the purpose of the project is selected the better.

The choices for purposes are:

- Basic research with the choice of all end-purpose categories
- Translational and applied research with the choice of all end-purpose categories
- Grouped category "Quality control including batch safety and potency testing" covering all end-purpose categories thereof
- Other efficacy and tolerance testing
- Grouped category 'Toxicity and other safety testing including pharmacology' covering all end-purposes and sub-categories thereof
- Grouped category 'Routine production' covering all end-purpose categories
- Protection of the natural environment in the interests of the health or welfare of human beings or animals
- Preservation of species
- Higher education
- Training
- Forensic enquiries
- Maintenance of colonies of genetically altered animals, not used in other procedures

A more detailed description of the project purposes eligible for selection may be found in [Annex III of the Decision \(Part A and Part B, B. Data input categories, points 10-21\)](#).

It is important to ensure the correct project purpose is selected in order to provide the public with an accurate representation of the reasons for animal use. As described earlier, keywords should be used to provide any further sub-categorisation, where appropriate.

### **Objectives and predicted benefits of the project**

**Objectives of the project: Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs)**

*Maximum length 2500 characters*

This section of the NTS is the first to be read by the interested public. Therefore, the overall goal of the project should be described in popular scientific (i.e. non-technical) language. Appropriate background information should be provided in order to give context to the research goal.

For example:

*'Duchenne muscular dystrophy (DMD) is a neuromuscular disease of humans that is characterised by severe muscle weakness, including the muscles of breathing. The main muscle of breathing, the diaphragm, is weakened in DMD with consequences for breathing and other functions of the respiratory*



*system including the ability to generate pressures in the chest that allow for effective cough and sneeze, which are important to clear the airways and help to protect against infections.'*

Next, the specific research questions that are being addressed should be described, explaining their relevance and why they are of interest.

For example:

*'There are still significant gaps in the understanding of respiratory system deficits in muscular dystrophy, particularly how these deficits progress as dystrophic disease advances. A major objective of this study is to examine respiratory system performance over the lifespan of mdx mice, a genetic animal model of DMD. It is thought that dietary interventions may improve muscle function and respiratory system performance in DMD. Therefore, the effectiveness of the dietary supplement and antioxidant (N-acetylcysteine) alone and in combination with the main steroid drug used in the treatment of DMD (prednisolone) will be determined by examining breathing and measures of respiratory system performance in mdx mice.'*

Remember; the main audience is the general public, not scientists.

**Potential benefits likely to derive from this project: What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished).**

*Max characters 2500*

While the 'objectives' section above describes the overall goal of the project and the relevance of the research questions being addressed, this section focuses on the potential impacts of the findings of the project. Benefits may be defined as the potential gains, insights into disease, or advances achieved for humans, other species, or the environment resulting from **this** project. It is worth highlighting that an 'increase of knowledge' may be a benefit *per se* but requires explanation in the context of scientific question being investigated e.g., 'the lack of a robust evidence-base on the precise mechanisms underpinning the pathology seen in Disease XYZ is a major obstacle in the development of new therapies for this condition'.

When describing the potential benefits, the following considerations should be incorporated in the details provided:

- a description of the potential benefits, ensuring that they are realistic;
- whether the potential benefits will be obtainable within this project, or if a further project will be required, e.g., projects which aim to establish a disease model, before using those models in another project to evaluate treatments for that model. In circumstances where a subsequent project is required for the realisation of longer-term benefits, it is important that the benefits described in the current NTS relate only to the specific project for which the NTS is being prepared.
- the potential advances in scientific knowledge which could be obtained, and the value of this knowledge;
- why these potential benefits are important;
- who will potentially benefit;
- an estimation of when any potential benefits may be expected to be realised;
- a description of how the benefits are likely to be realised (e.g. by scientists, the pharmaceutical industry, clinicians, human patients or animals).

For example:

*'The short-term benefit of this study is that it should contribute to scientists' understanding of the progression of respiratory system impairment and failure in dystrophic disease. A significant potential longer term benefit of the study is the investigation of a new potential therapy, with a view to prolonging life expectancy by improving respiratory system performance in dystrophic disease.'*

The means by which negative results will be addressed, and whether they could be beneficial, should also be described if applicable. It is important for the credibility of NTS that the description of potential benefits that may be achieved is realistic and not overstated. For example, the basic research project example 2 in Appendix II states:

*'A possible benefit of the project is finding a cure for human cancers'.*

This is a clear overstatement of the possible benefits that could be achieved in a basic research study, and therefore is not accurate or appropriate.

### **Predicted harms**

**In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures**

*Max characters 2500*

Describe here the single- or multi-step procedures that will typically be used, for each animal or group of animals. A procedure is performed to answer a particular scientific question. Procedures may be simple or complex, depending on the scientific question being addressed. They may comprise only a single step (e.g. withdrawal of blood), but much more commonly necessitate multiple steps performed in a particular sequence.

Users completing an NTS should describe the interventions that comprise the procedures involved in an appropriate level of detail that will enable the reader to gain a good understanding of what is being done to each animal/group of animals. In some instances, for example in the case of simple procedures made up of only a small number of interventions, it will be feasible to describe each intervention. However, in other instances, such as in the case of very complex procedures that comprise many interventions, it may be necessary to group interventions at a higher level, albeit the overall description of what each animal/group of animals will undergo should still be clear and readily apparent.

By way of example, in a pharmaceutical project that has the aim of understanding the distribution of a test substance within the tissues and organs of the body, there may only be one procedure performed, and that could be a single-step procedure. This single-step procedure could consist of one subcutaneous injection of a test substance, followed by killing of the animal using a method approved in Annex IV to the [Directive](#), within a defined period after the subcutaneous injection. In this example, the single intervention performed (subcutaneous injection) constitutes a procedure. Provided the test substance administered does not cause any pharmacological adverse effects, the duration of this procedure is limited to the time it takes to administer the substance via subcutaneous injection, e.g. one minute.

In contrast, in example 1 (Basic research) of the illustrative examples of NTS included in Appendix II, the procedure described is a multi-step procedure necessitating a number of separate interventions, which must be performed in a certain sequence, in order to answer a specific scientific question.

In this example, the multi-step procedure that mice will undergo consists of:

1. Injection of test substances,
2. A battery of behavioural tests (including the forced-swim test) to measure sociability, anxiety, depressive-like behaviour and learning ability,
3. A cranial surgery to implant a device in the brain to facilitate the recording of brain signals by wireless electroencephalogram,
4. A Magnetic Resonance Imaging brain scan performed under general anaesthesia from which the animal is not awakened.

The maximum possible duration of the procedure in this case is 24 days.

For more information on the definition of a procedure, please refer to the [2011 Working document on specific articles in Directive 2010/63/EU](#).

**Expected impacts/adverse effects on the animals: What are the expected impacts/ adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?**

*Max characters 2500*

Here, the impact of the whole experimental procedure (single or more often multi-step, and which lasts in most instances for the entire duration of the animal's time on study) should be summarised (i.e. some interventions maybe repeated, etc.). So, this part will address all possible impacts/adverse effects, including cumulated harms if any, of each procedure.

For example:

*'Animals may experience transient pain at the site of injection and these injections will be repeated on several occasions. Animals may experience fatigue or distress when undergoing the swim test, and this could last for up to ten minutes.*

*Animals will then undergo surgery to have a device implanted to enable EEG recording, and this will result in postoperative pain. There may be individual animal variation in how long animals experience this pain, but it is expected to last for approximately three days on average. There is also a slight risk of haemorrhage or infection of the surgical site, however if either of these occur animals will be euthanised immediately, therefore they are not expected to experience these effects for an extended duration.'*

**What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?**

Species – please complete one line per species.

Please note that the submission will not be valid unless at least one species is selected.

The drop-down menu includes a selection entitled 'non-specified mammal'. Selection of this 'species' option is strictly limited to those exceptional circumstances where naming the species would enable identification of the research group performing the study, or the establishment at which the authorised work is to be performed. Use of this species category will be closely monitored by competent authorities,

and in instances where it has been selected inappropriately, applicants will be required to revise the submission.

### **Estimated numbers per severity**

When completing this section of the NTS for each species used, a value must be entered for each severity classification (otherwise submission of the NTS will be rejected by the EC database). So for example, if 100 mice are to be used in a project, and all 100 are expected to reach a cumulative severity of mild, values of 0 must be entered for each of the three remaining severity categories.

It is important to emphasise that the information captured in this section of the template should relate to the prospective *cumulative* (within the procedure) suffering that each animal or group of animals is expected to reach. This is as a result of both the intended impacts of procedures, and any adverse effects that are expected, throughout the duration of the procedure(s) captured under the project authorisation to which the NTS relates. Therefore, the NTS should not describe severities as assigned to each discrete procedure *per se*, but rather the overall maximum severities that each animal/group of animals (of each species used) is realistically anticipated or likely to experience.

Please note that for each species there should only be one line entry describing the expected distribution of severities animals will experience resulting from all procedures for that species.

Definitions of each of the four severity categories are provided below (as per [Annex VIII](#) of the [Directive](#). Additional information on severity can be found in the [EC Severity Assessment Framework document](#) and also in chapter 'Factors to take into consideration in assessing harms' of the [EC Working Document on Project Evaluation and Retrospective Assessment](#)

#### **Non-recovery:**

Procedures which are performed entirely under general anaesthesia from which the animal shall not recover consciousness shall be classified as 'non-recovery'. If interventions other than those directly related to anaesthetising the animal are performed before general anaesthesia (e.g. injection of a substance related to the experimental goal, rather than the anaesthesia), another severity classification must be assigned.

#### **Mild:**

Procedures on animals as a result of which the animals are likely to experience short-term mild pain, suffering or distress, as well as procedures with no significant impairment of the well-being or general condition of the animals shall be classified as 'mild'.

#### **Moderate:**

Procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress as well as procedures that are likely to cause moderate impairment of the well-being or general condition of the animals shall be classified as 'moderate'.

#### **Severe:**

Procedures on animals as a result of which the animals are likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress as well as procedures, that are likely to

cause severe impairment of the well-being or general condition of the animals shall be classified as 'severe'.

### **Fate of animals kept alive**

**What will happen to the animals kept alive at the end of the procedure? Please complete 'Fate of animals kept alive' tab if relevant (i.e. if any animals are to be reused/returned to habitat/husbandry system/rehomed at the end of their time on study).**

#### *Estimated number to be reused*

Reuse is a term to indicate the subsequent use of an animal that has already completed a procedure (or series of procedures/techniques) for a particular scientific purpose. Article 16 of the [Directive](#) defines reuse as a use 'when a different animal on which no procedure has previously been carried out could also be used'. Article 16 also defines the circumstances under which it is considered acceptable to reuse an animal.

For further guidance on the definition of reuse, please see the relevant sections of [EC Working document on specific articles in Directive 2010/63/EU](#) and [Annex III of the Decision \(Part B, Section B, point 2.2 onwards\)](#).

#### *Estimated number to be returned to habitat/husbandry system*

An example of a project in which animals could be returned to habitat is a wildlife conservation study, in which animals are captured in their normal habitat, held for a short space of time to enable them to be tagged with a tracking device, and then immediately released at the site of capture. An example of a project in which an animal could be returned to a husbandry system is an agricultural study investigating the nutritional properties of various types of feedstuffs. Cattle living on a commercial farm could be enrolled on study, fed a particular diet for a period of time and have a number of blood samples taken to assess certain metabolic parameters. When the project ends, these cattle would be considered no longer 'on study', and would be classified as having been returned to their normal husbandry system.

#### *Estimated number to be rehomed*

Rehoming is the movement of an animal used for scientific purposes from an authorised breeder/supplier/user establishment to any other place that is not a breeder/supplier/user establishment authorised under the scientific animal protection legislation. There are a wide range of destinations for rehomed animals such as private homes (e.g. for companion animals), privately owned stables, aquariums etc.

### **Reasons for the planned fate of the animals after the procedure**

**Please provide reasons for the planned fate of the animals after the procedure.**

*Max characters 2500*

In this section, the reasons underpinning the planned fate of **all** animals (i.e. not only those that are reused/returned to habitat/husbandry/rehomed) intended to be used on the project should be described. If it is planned to reuse animals, return them to habitat/husbandry system, or rehome them, please provide justification as to why this is the most appropriate option for these animals.

If animals are planned to be killed during the project, or upon conclusion of the project, then please explain briefly why this is necessary (i.e. why reuse/returning to habitat/husbandry/rehoming is not possible). For example, in cases where animals are being killed in order to harvest their tissues and organs for histology or other analysis, a brief explanation of what this analysis is, and why it is required to achieve the objectives of the study should be provided.

## **Application of the Three Rs**

### **1. Replacement**

**State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project**

*Max characters 2500*

Article 4 of the [Directive](#) states that *'wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure.'* It should be demonstrated in this section that potential alternatives to the use of live animals have been thoroughly explored, and that no suitable alternatives have been identified.

Explain and demonstrate why there is no alternative to the use of animals in order to achieve the specific objectives of this project.

For example:

*'Complex neurological processes such as learning and memory and social interactions involve several different regions of the brain and rely on intact connections between these regions. This project proposes to study these processes in a mouse model of Fragile X Syndrome and to investigate the underlying brain mechanisms; this necessitates observation of actual behaviour of a living organism.'*

Explain what animal alternatives (partial and/or full Replacement) were considered before reaching the point where animal use has become necessary. These may include *in silico*, *in vitro* or *ex vivo* approaches. If non-animal methods have already been used (e.g. in preliminary work) or will be integrated with the proposed *in vivo* studies, this information should also be included.

For example:

*'Other alternatives have been considered including computational models and ex vivo organoids (e.g. brain on a chip), however, the core aim of this project is to study changes in behaviour, which is not possible in the above-mentioned alternatives.'*

An example of a poor response to this question is:

*'The project by nature should include laboratory animals, as the main objective is to understand the existence of individuals in a population with cancer mutations.'*

This statement does not explain why animals are necessary to achieve the scientific objectives of the project, nor does it acknowledge the non-animal alternatives that are available in oncology research.

## 2. Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

*Max characters 2500*

The information provided in this section should firstly demonstrate how the appropriate number of animals to be used was determined, consistent with the project's objectives.

For example:

*'Detailed statistical calculations were performed to determine the appropriate number of animals for this project. The calculations were informed by studies reported in the literature, which used similar behavioural tests using Fmr1 KO mice. The number of animals to be used will allow scientifically robust data to be generated.'*

Secondly, it should document every step that was taken during design of the project to reduce the number of animals in procedures (e.g. checking and validating the importance and number of each control animal/ group of animals, optimising study design to maximize statistical power (follow-up, longitudinal studies etc.).

For example:

*'Individual animals will undergo multiple (up to mild severity) behavioural tests, as opposed to different animals being used for each test, so that the maximum amount of data is obtained from each animal. This reduces the overall animal numbers required. Furthermore, post mortem analyses will be conducted on the tissue from all animals to ensure the maximum possible information is gathered from each animal. The animal numbers and experimental design for this project were also reviewed and approved by an experienced biostatistician.'*

Additionally, practices that will in part replace the use of animals and that may also contribute to Reduction should be captured here, if relevant.

For example:

*'a method where we can study how the hens' white blood cells phagocytose ("eat up") rubella bacteria will be used. This method has been developed by the research group in a previous study and can be seen as a model to partially replace the use of animals in infection studies because it reflects the individual's ability to defend against rubella bacteria.'*

The use of statistical terminology that would not be expected to be understood by a layperson is not appropriate and should be avoided.

For example:

*'Statistical analysis will be carried out at all stages of the project...mainly by means of a 'two sample independent t-test' analysis.'*

### 3. Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.**

*Max characters 2500*

Consideration must be given to all of the negative effects that animals may experience as a result of procedures, and the measures that will be implemented to minimise these effects should be clearly described. In general, it is not appropriate to describe provisions that are essential legislative requirements (e.g. environmental enrichment, access to appropriate veterinary care, competency to perform procedures etc.) in response to this question. It is however important to clearly outline all of the specific and tailored measures which will be taken to alleviate the harms inherent to the particular procedure(s) being performed. Therefore, the strategies being employed to address the expected impacts/adverse effects of procedure(s) on the animals (for example habituation, analgesia, anaesthesia, special diets, acute/intensive monitoring etc.) should be detailed.

For example:

*'Mice will be handled appropriately and they will be interacted with frequently, which will reduce the level of stress during procedures. Anaesthesia will be used for the MRI and the surgical implant of wireless EEG caps. Animals will receive pain relief peri- and post-operatively to minimise pain and suffering. In order to safeguard welfare, animals will be monitored and scored frequently using animal welfare score sheets to ensure that no animal exceeds a strictly pre-determined level of distress, and humane endpoints will be implemented immediately if any animal is found to be experiencing unexpected adverse effects.'*

The means by which emerging refinement techniques will be adopted throughout the lifetime of the project should also be described, where appropriate.

For example:

*'Regular literature reviews will be performed throughout the duration of the project to ensure that procedures being used are as refined as possible and remain in line with good practice recommendations. The research group will also maintain good lines of communication with the establishment's AWB, information officer, and designated veterinarian with a view to implementing any refinement opportunities (e.g. in relation to animal monitoring, humane endpoints, refinement of surgical techniques, analgesic regimes etc.) that emerge during the course of this study.'*

For regulatory testing, it is not sufficient to refer to regulatory guidance to demonstrate refinement, for example:

*'All activities will be performed in accordance with ISO 10993 Part 10: "Skin irritation and sensitisation testing.'*

While this infers that studies will be carried out in accordance with good laboratory practice, a lay audience would not be expected to be familiar with regulatory guidance and standards and so refinement measures should be explained in clear and simple language.



## **Explain the choice of species and the related life stages.**

*Max characters 2500*

The scientific justification of choice of species and the related life stages should be summarised here. Explain why the species and related life stage chosen is the most appropriate and refined to achieve the stated objectives of the project.

For example:

*'For these studies mice have been chosen, specifically a strain of mouse that is genetically altered to have a similar molecular phenotype (characteristics) as found in patients with FXS (e.g. the absence of functional FMR1 protein). Choosing mice will allow for the measurement of behavioural changes that occur in these animals as a result of the Fmr1 mutation. Therefore, these mice are the most appropriate model to achieve the aims of this study. Juvenile mice will be used in this study as behavioural and cognitive impairments are observed in human children with FXS.'*

## **Project selected for retrospective assessment**

This section will be filled in by the competent authority tasked with the project evaluation.

Only those Member States that have introduced the requirement in their national legislation transposing the [Directive](#) to specify in the NTS whether a project is to undergo a RA, will be required to fill in this section.

The deadline for completing a retrospective assessment should be given. It should reflect a time point after the completion of the project that allows a realistic assessment of whether the expected benefits have been attained.

Retrospective assessment is compulsory to all projects containing severe procedures and/or using non-human primates. In addition, competent authority during the project evaluation should decide which other projects should undergo a retrospective assessment. In this case, the reasons why a project is selected for retrospective assessment should be given.

**Appendix I – Template for the non-technical project summary**

*[the template will be provided here at a later stage using a link to allow viewing of dropdown menus]*

DRAFT

## Appendix II – Illustrative examples of completed non-technical project summaries

### 1. BASIC RESEARCH

#### Example 1 (good quality)

|   |  |
|---|--|
| <b>Title of the project</b>   | A research study to investigate novel drugs for the treatment of the genetic disorder Fragile X syndrome   |
| <b>Duration of project</b><br>(in months)   | 36 months  |
| <b>Keywords</b>   | <i>Fmr1</i> gene knockout; GSK3beta inhibitors; learning difficulties; behavioural disorder; autism features   |
| <b>Purpose of project</b><br>(multiple choices possible)  | Basic research - nervous system  |
| <b>Objectives and predicted benefits of the project</b>   |  |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs)   | <p>Fragile X Syndrome (FXS) is a rare genetic disorder in humans, which affects predominately males. FXS results in a range of developmental problems including learning disabilities and cognitive impairment. Children with FXS may also have anxiety, hyperactive behaviour (fidgeting or impulsive actions), and attention deficit disorder (an impaired ability to maintain attention and difficulty focusing on specific tasks). One third of those with FXS have features of autism spectrum disorder, affecting communication and social interaction.</p> <p>Seizures occur in 15% of males and 5% of females with FXS. The mechanisms underlying these symptoms are not clear. There is no cure for FXS but there are some treatments that can help to manage symptoms. FXS is associated with a mutation in the <i>Fmr1</i> gene, preventing production of a functional protein. The <i>Fmr1</i> knockout (KO) mouse has similar molecular and behavioural characteristics as FXS patients. Microtubules (structural proteins that support cell function) may be altered in <i>Fmr1</i> KO mice and FXS patients. Thus, the <i>Fmr1</i> KO mouse will be used to model social deficits and anxiety-like behaviours, and to determine the efficacy (the ability to produce the desired result) of a novel compound on cognitive and social symptoms in the <i>Fmr1</i> KO mouse. Changes in microtubules will be measured in <i>Fmr1</i> KO mice to assess their potential as a biomarker for FXS (i.e. a measurable indicator of the presence or severity of the disease).</p> |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where | In the short-term, the findings from this study will benefit the scientific and medical community studying the mechanisms underlying FXS and attempting to develop new treatments. In the longer term, understanding how the <i>Fmr1</i> gene contributes to neurodevelopment (the process by which the physiological and psychological growth of the brain occurs) and how it can impact on learning and cognition will provide new insights into this disorder. Investigation of microtubules in FXS as a biomarker may generate potential new targets for therapies   |

|  |   |                                |  |             |                                       |               |
|--|---|--------------------------------|--|-------------|---------------------------------------|---------------|
| applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished).                      | and provide clinicians with a quantitative measure of syndrome severity. Furthermore, the effect of novel compounds that can alter microtubules will be tested to see if they can improve cognitive and social symptoms in the <i>Fmr1</i> KO mouse, which may ultimately have implications for future patient treatment. Together, these studies may reveal a novel therapeutic and biomarker for improving quality of life in FXS.  |                                |  |             |                                       |               |
| <b>Predicted harms</b>   |   |                                |  |             |                                       |               |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.                              | Mice will undergo a multi-step procedure consisting of a number of different interventions over the course of the project. All animals will receive novel drugs via injection. Animals will then undergo different behavioural tests to measure social interactions, anxiety, and learning and memory. Adverse effects are not expected as a result of these tests. Some animals may be tested for depressive-like behaviour, and they will be required to swim for this test. Mice will then undergo a surgery to have a device implanted in their brain to facilitate the recording of brain signals by wireless EEG (a measure of brain wave activity), followed by a Magnetic Resonance Imaging (MRI) brain scan from which they will not be awakened. The maximum possible duration of the procedure is 24 days. |                                |  |             |                                       |               |
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects? | Animals may experience transient pain at the site of injection and these injections will be repeated on several occasions. Animals may experience fatigue or distress when undergoing the swim test, and this could last for up to ten minutes.<br><br>Animals will then undergo surgery to have a device implanted to enable EEG recording, and this will result in postoperative pain. There may be individual animal variation in how long animals experience this pain for, but it is expected to last for approximately three days on average. There is also a slight risk of haemorrhage or infection of the surgical site, however if either of these occur animals will be euthanised immediately, therefore they are not expected to experience these effects for an extended duration.                      |                                |  |             |                                       |               |
| What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?                        | <b>Species</b>  | <b>Estimated total numbers</b> | <b>Estimated numbers per severity</b>                              |             |                                       |               |
|  |   |                                | <b>Non-recovery</b>  | <b>Mild</b> | <b>Moderate</b>                       | <b>Severe</b> |
|  | Mice  | 540                            | 0  | 0           | 540                                   | 0             |
|  |   |                                |  |             |                                       |               |
|  |   |                                |  |             |                                       |               |
|  |   |                                |  |             |                                       |               |
| What will happen to the animals kept alive at the end of the procedure? <sup>5, 6</sup>  | <b>Estimated number to be reused</b>  |                                | <b>Estimated number to be returned to habitat/husbandry system</b> |             | <b>Estimated number to be rehomed</b> |               |
| Please provide reasons for the planned fate of the   | At the end of the study, all animals will be humanely euthanised for tissue collection and analysis.  |                                |  |             |                                       |               |

|   |   |
|---|---|
| animals after the procedure.  |   |
| <b>Application of the Three Rs</b>  |   |
| <p><b>1. Replacement</b><br/>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.</p>   | <p>Complex neurological processes such as learning and memory and social interactions involve several different regions of the brain and rely on intact connections between these regions. This project proposes to study these processes in a mouse model of FXS and to investigate the underlying brain mechanisms; this necessitates observation of actual behaviour of a living organism. Other alternatives have been considered including computational models and <i>ex vivo</i> tissue methods, however, the core aim of this project is to study changes in behaviour, which is not possible in the above-mentioned alternatives. As such, a non-animal approach is not appropriate for this project, as the complex model required cannot be replicated through <i>in vitro</i> (non-animal) techniques.</p>  |
| <p><b>2. Reduction</b><br/>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.</p> | <p>Detailed statistical calculations were performed to determine the appropriate number of animals for this project. The calculations were informed by studies reported in the literature, which used similar behavioural tests using <i>Fmr1</i> KO mice. The number of animals to be used will allow scientifically robust data to be generated. Individual animals will undergo multiple (up to mild severity) behavioural tests, as opposed to different animals being used for each test, so that the maximum amount of data is obtained from each animal. This reduces the overall animal numbers required. Furthermore, post mortem analyses will be conducted on the tissue from all animals to ensure the maximum possible information is gathered from each animal. The animal numbers and experimental design for this project were also reviewed and approved by an experienced biostatistician.</p>  |
| <p><b>3. Refinement</b><br/>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.</p>  | <p>Mice will be handled appropriately and they will be interacted with frequently, which will reduce the level of stress during procedures. Anaesthesia will be used for the MRI and the surgical implant of wireless EEG caps. Animals will receive pain relief peri- and post-operatively to minimise pain and suffering. In order to safeguard welfare, animals will be monitored and scored frequently using animal welfare score sheets to ensure that no animal exceeds a strictly pre-determined level of distress, and humane endpoints will be implemented immediately if any animal is found to be experiencing unexpected adverse effects.</p> <p>Opportunities to refine the procedure will be continuously explored and implemented where possible during the lifetime of the project. Regular literature reviews will be performed throughout the duration of the project to ensure that the procedures being used are as refined as possible and remain in line with good practice recommendations. The research group will also maintain good lines of communication with the establishment's AWB, information officer, and designated veterinarian with a view to implementing any refinement opportunities (e.g. in</p> |

|  |  |
|--|--|
|  | relation to animal monitoring, humane endpoints, refinement of surgical techniques, analgesic regimes etc.) that emerge during the course of this study.   |
| Explain the choice of species and the related life stages. | For these studies mice have been chosen, specifically a strain of mouse that is genetically altered to have a similar molecular phenotype (characteristics) as found in patients with FXS (e.g. the absence of functional FMR1 protein). Choosing mice will allow for the measurement of behavioural changes that occur in these animals as a result of the <i>Fmr1</i> mutation. Therefore, these mice are the most appropriate model to achieve the aims of this study. Juvenile mice will be used in this study as behavioural and cognitive impairments are observed in human children with FXS. |

**This is considered a good quality NTS because:**

- **Language is clear concise and understandable to a layperson**
- **Acronyms and scientific terms are defined**
- **It is anonymous**
- **Clear informative keywords specific to this research are provided. Both scientific and lay keywords are used – these are useful to both the public and scientists** (*this may need to be revised based on NCP feedback*)
- **Objectives are clearly described**
- **Benefits are clearly described but not overstated**
- **All of the potential harms and their expected duration are listed**
- **Information on implementation on each of the 3Rs is provided, including:**
  - **Clear information on why replacement is not possible to achieve the scientific objectives**
  - **Details are provided on the Reduction measures being applied**
  - **Comprehensive information on Refinement measures is included**
- **Information is provided to justify the species and life stage used**

**Example 2 (poor quality)**

|   |   |
|---|---|
| <b>Title of the project</b>   | Survival of zebrafish with genetically modified cancer genes in response to various stressors   |
| <b>Duration of project</b><br>(in months)   | 60 months   |
| <b>Keywords</b>   | Stress; temperature; physical selection; knock-out; genetic modification  |
| <b>Purpose of project</b><br>(multiple choices possible)  | Basic research -<br>other basic research  |
| <b>Objectives and predicted benefits of the project</b>   |   |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | The project's main objective is to understand the existence of mutations related to cancer in the general population, and why these are not excluded by physical selection.                             |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). | A possible benefit of the project is finding a cure for human cancers. This research will help to understand why cells with cancer-causing mutations are not effectively neutralised by the human body. |
| <b>Predicted harms</b>  |   |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.   | Mutant fish will be bred.   |
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?  | The feeling of pain and suffering in the fish will be minimised, since MS222 will be used for anaesthesia and euthanasia, which is a globally accepted method.<br><br>Severity: Mild or medium.         |

|   |  |  |                                       |                                       |                 |               |
|---|--|--|---------------------------------------|---------------------------------------|-----------------|---------------|
| <p>What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?</p>  | <b>Species<sup>4</sup></b>   | <b>Estimated total numbers</b>                                     | <b>Estimated numbers per severity</b> |                                       |                 |               |
|   |  |  | <b>Non-recovery</b>                   | <b>Mild</b>                           | <b>Moderate</b> | <b>Severe</b> |
|   | Zebrafish  | 120  | 120                                   | 0                                     | 0               | 0             |
|   |  |  |                                       |                                       |                 |               |
|   |  |  |                                       |                                       |                 |               |
| <p>What will happen to the animals kept alive at the end of the procedure?<sup>5,6</sup></p>  | <b>Estimated number to be reused</b>   | <b>Estimated number to be returned to habitat/husbandry system</b> |                                       | <b>Estimated number to be rehomed</b> |                 |               |
|   |  | 120  |                                       |                                       |                 |               |
| <p>Please provide reasons for the planned fate of the animals after the procedure.</p>  | <p>At the end of the project, most of the animals will be euthanised. A limited number of animals may be maintained for future projects.</p>   |  |                                       |                                       |                 |               |
| <b>Application of the Three Rs</b>  |  |  |                                       |                                       |                 |               |
| <p><b>1. Replacement</b><br/>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.</p>   | <p>The project by nature should include laboratory animals, as the main objective is to understand the existence of individuals in a population with cancer mutations.</p>   |  |                                       |                                       |                 |               |
| <p><b>2. Reduction</b><br/>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.</p> | <p>Statistical analysis will be carried out at all stages of the project in order to determine the lowest number of animals with the best research result is always used, mainly by means of a 'two sample independent t-test' analysis.</p>   |  |                                       |                                       |                 |               |
| <p><b>3. Refinement</b><br/>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain</p>   | <p>Animals will be handled only by trained personnel. <i>The</i> methods to be used are internationally appropriate for these type of experiments. The use of anaesthetic MS222 and a modern animal unit, where the conditions for keeping animals are ideal, ensure the least possible suffering of the animals, but also animal welfare.</p> |  |                                       |                                       |                 |               |



|  |                 |
|--|-----------------|
| management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project. |                 |
| Explain the choice of species and the related life stages.   | Adult zebrafish |

This is considered a poor quality NTS because:

- **Keywords are not considered very informative**
- **“Basic Research - Other basic research” has been selected as the project purpose. “Basic research – Oncology” would be more appropriate in this case**
- **Predicted harms:**
  - **The only procedure described is the breeding of mutant fish, however, under adverse effects it is stated that anaesthesia (MS222) will be used. Therefore, it would appear not all procedures/interventions have been captured**
  - **No adverse effects have been described – information on anaesthesia is not appropriate in this section**
- **120 fish have been classified as non-recovery. This does not agree with the other information provided**
- **It is stated that 120 fish will be returned to husbandry. This does not agree with the other information provided. The figures are also inconsistent with the field below where it is stated that the majority of fish will be euthanised.**
- **There is limited information on the implementation of the 3Rs:**
  - **There is no information on available alternatives that were considered, and the use of animals has not been adequately justified**
  - **Detailed information on statistical tests (“two sample independent t test”) should not be included as it is not helpful lay people**
  - **It is stated that “statistical analysis will be carried out” – no evidence of *a priori* sample size calculations**
  - **No information has been provided on “practices that will be used throughout the project to minimise the number of animals used”**
  - **No information is provided on “mechanisms to take up emerging refinement techniques during the lifetime of the project”**
- **No information is provided to justify the species and the life stage of the animals to be used**

## 2. TRANSLATIONAL AND APPLIED RESEARCH

### Example 3 (good quality)

|   |  |
|---|--|
| <b>Title of the project</b>   | Effects of an amino acid supplement on breathing function in a mouse model of muscular dystrophy: research relevant to boosting respiratory function in neuromuscular disease  |
| <b>Duration of project</b><br>(in months)   | 60 months  |
| <b>Keywords</b>   | <i>Mdx</i> gene; progressive muscle degeneration; <i>dystrophin</i> ; respiratory muscle weakness; N-acetylcysteine supplementation  |
| <b>Purpose of project</b><br>(multiple choices possible)  | Translational and applied research - human musculoskeletal disorders   |
| <b>Objectives and predicted benefits of the project</b>   |  |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | Duchenne muscular dystrophy (DMD) is a neuromuscular disease of humans that is characterised by severe muscle weakness, including the muscles of breathing. The main muscle of breathing, the diaphragm, is weakened in DMD with consequences for breathing and other functions of the respiratory system including the ability to generate pressures in the chest that allow for effective cough and sneeze, which are important to clear the airways and help to protect against infections. There are still significant gaps in the understanding of respiratory system deficits in muscular dystrophy, particularly how these deficits progress as dystrophic disease advances. A major objective of this study is to examine respiratory system performance over the lifespan of <i>mdx</i> mice, a genetic animal model of DMD. It is thought that dietary interventions may improve muscle function and respiratory system performance in DMD. Therefore, the effectiveness of the dietary supplement and antioxidant (N-acetylcysteine) alone and in combination with the main steroid drug used in the treatment of DMD (prednisolone) will be determined by examining breathing and measures of respiratory system performance in <i>mdx</i> mice. |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits | DMD develops in approximately 1 out of every 3300 male births in humans worldwide. It is important to increase knowledge of the effects of dystrophic disease on respiratory system function, given that patients with DMD die prematurely due to respiratory and cardiac failure. Lung function is at its best when patients are in their mid-teens, with various breathing abilities decreasing steadily thereafter. The average life expectancy of patients with DMD is in the mid to late 20s. There are many unknowns in respect of respiratory system performance across the lifespan in DMD and animal models of the disease. The short-term benefit of this study is that it should contribute to scientists' understanding of the progression of respiratory system impairment and failure in dystrophic disease. A significant potential longer term benefit of the study is the investigation of a new potential therapy, with a view   |

|  |  |  |                                       |             |                 |               |
|--|--|--|---------------------------------------|-------------|-----------------|---------------|
| (which may accrue after the project is finished).  | to prolonging life expectancy by improving respiratory system performance in dystrophic disease.   |  |                                       |             |                 |               |
| <b>Predicted harms</b>   |  |  |                                       |             |                 |               |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.                              | <p>Each mouse will be used in a single procedure, which consists of a number of different interventions. Genetically altered <i>mdx</i> mice will be bred; for the most part, these mice live a normal life and display no obvious signs of illness or distress. Breathing and metabolism will be measured in conscious, freely mobile mice in custom-made, specialised chambers. Animals tolerate the pressure changes associated with this test very well, and no adverse effects are expected. The majority of mice will receive a low dose of a steroid drug by injection once weekly for up to one year.</p> <p>Mice will receive a dietary supplement in their drinking water. Some mice will be put under anaesthesia, and pressure within the chest of the animal will be measured. A different group of mice will be put under anaesthesia and the pressure in the food pipe and in the stomach will be measured. The difference between these pressures is known as the trans-diaphragmatic pressure, which is an index of diaphragm function in the living animal. A third group of mice will be placed under anaesthesia and blood will be collected for analysis. Animals will remain on study for up to 16 months.</p> |  |                                       |             |                 |               |
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects? | <p>It is not expected that mice will experience adverse effects as a result of their genetic alteration, or due to the measurement of breathing and metabolism.</p> <p>The majority of mice will undergo repeated injections. Mice will experience mild pain at each injection, but since this injection will be repeated at such a high frequency (once weekly for up to one year), there will be cumulative effects, and these mice may experience moderate distress as a result. Measurements of breathing and metabolism will be carried while mice are under general anaesthesia from which the animals will not be recovered.</p>  |  |                                       |             |                 |               |
| What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?                        | <b>Species</b>   | <b>Estimated total numbers</b>                                     | <b>Estimated numbers per severity</b> |             |                 |               |
|  |  |  | <b>Non-recovery</b>                   | <b>Mild</b> | <b>Moderate</b> | <b>Severe</b> |
|  | Mice   | 3231   | 0                                     | 204         | 3027            | 0             |
|  |  |  |                                       |             |                 |               |
|  |  |  |                                       |             |                 |               |
| What will happen to the animals kept alive at the end of the procedure?  | <b>Estimated number to be reused</b>   | <b>Estimated number to be returned to habitat/husbandry system</b> | <b>Estimated number to be rehomed</b> |             |                 |               |
| Please provide reasons for the planned fate of the   | Any animals that are not euthanised whilst anaesthetised for measurement of breathing and metabolism throughout the study will   |  |                                       |             |                 |               |

|   |  |
|---|--|
| animals after the procedure.  | be euthanised at the end of the study and tissues collected for further analysis.  |
| <b>Application of the Three Rs</b>  |  |
| <p><b>1. Replacement</b><br/>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.</p>   | <p>This project will carry out novel research for which the information is not already available in the literature. Although replacement via non-animal alternatives such as cell lines is an invaluable asset to research, cell lines cannot replicate the complex integrative physiology present in the mouse, which closely aligns to that of humans. Due to the high level of complexity of the body systems monitored (respiratory and musculoskeletal) and their complex integration, there are too many unknowns to create reliable computer generated models. Thus, there are no non-animal models that can be utilised for these studies.</p>   |
| <p><b>2. Reduction</b><br/>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.</p> | <p>This project has been designed to use the minimum numbers of animals, while ensuring scientifically relevant results can be obtained and the objectives of the study can be achieved. Statistical calculations were carried out based on the results of similar studies in this area. When possible, organs obtained from mice will be preserved and stored for subsequent tests, maximising the data obtained from each animal. The required number of mice needed for this study is reduced by recording as many parameters as feasible from a single mouse, without compromising animal welfare.</p>   |
| <p><b>3. Refinement</b><br/>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.</p>  | <p>Body weights will be recorded at the start of every procedure. During drug and diet supplementation, body weights will be recorded once per week. If the animals exhibit signs of stress (e.g. weight loss, dishevelled appearance etc.) they will be removed from the study and humanely euthanised. The dose of drug to be administered to the animals is low compared to other studies, thereby, reducing the potential for adverse effects. An animal welfare score sheet will be used to monitor the health and welfare of the animals after interventions.</p> <p>Regular literature reviews will be performed to identify any emerging refinements that could be applied to this project. Researchers will also attend 3Rs-related conferences to learn about new refinement opportunities and best practice guidelines.</p> |
| <p>Explain the choice of species and the related life stages.</p>   | <p>Mice have a long history in terms of usage in biomedical research and consequently a large volume of information is available on this species, especially in respiratory diseases. Mice have been carefully bred to</p>   |

|  |  |
|--|--|
|  | <p>produce genetically similar animals that reduce variation. Mice share 85% of their genetic make-up with humans, making them a suitable model for research relevant to human disease. The <i>mdx</i> mouse model of DMD has played a vital role in understanding muscular dystrophy. There are certain characteristics of this model, which imitate DMD in humans e.g. breathing muscle weakness, reduced muscle elasticity, structural alterations and inflammation.</p> <p><i>Mdx</i> mice will be used from birth to adulthood (up to 16 months of age) in order to understand the course of DMD throughout the lifespan.</p> |
|--|--|

**This is considered a good quality NTS because:**

- **Language is clear concise and understandable to a layperson**
- **Acronyms and scientific terms are defined**
- **It is anonymous**
- **Clear informative keywords specific to this research are provided**
- **Objectives are clearly described**
- **Benefits are clearly described but not overstated**
- **All of the potential harms and their expected duration are listed**
- **Information on implementation on each of the 3Rs is provided, including:**
  - **Clear information on why replacement is not possible to achieve the scientific objectives**
  - **Details are provided on the Reduction measures being applied**
  - **Comprehensive information on Refinement measures is included**
- **Information is provided to justify the species and life stage used**

#### Example 4 (poor quality)

|   |  |
|---|--|
| <b>Title of the project</b>   | Studies of immunological mechanisms in vaccination against rubella in laying hens  |
| <b>Duration of project</b><br>(in months)   | 90 months  |
| <b>Keywords</b>   | Chickens; rubella; immune system   |
| <b>Purpose of project</b><br>(multiple choices possible)  | Translational and applied research - animal diseases and disorders   |
| <b>Objectives and predicted benefits of the project</b>   |  |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | The overall aim of the project is to increase knowledge about how the mechanisms in the immune system of chickens reacts when they are vaccinated against rubella. This includes how the protection against disease varies between individual chickens and flocks, and how long the protection can be expected to last.  |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). | The disease rubella is a bacterial infection, which may affect a wide range of animal species, including chickens. In laying flocks, the disease causes animal suffering and economic losses caused by acute outbreaks that result in high mortality and reduced egg production. In other animal species, vaccination protects against rubella by stimulating the production of antibodies. In the case of hens, the importance of antibodies is less clear, as the research is very limited. This study will provide important basic knowledge on the functioning of a rubella vaccine in hens, which is currently lacking. For example, it will test how long the protection continues after vaccination. This will help to determine the usefulness of the current vaccination strategy on the prevention of disease in laying hens, including how high and variable antibody levels are after vaccination and how long they are being maintained. This knowledge will make it possible to make informed recommendations for the vaccination of laying hens in order to prevent outbreaks of rubella. |
| <b>Predicted harms</b>  |  |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.   | Laying hens in flocks will be blood sampled and then returned to their normal environment.   |
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?  | During the blood sampling, the birds are restrained and also feel a sting from the needle. Most birds lie quiet and still during sampling and do not react to the needle stick itself.   |

|   |  |  |                                       |             |                 |               |
|---|--|--|---------------------------------------|-------------|-----------------|---------------|
| <p>What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?</p>  | <b>Species</b>   | <b>Estimated total numbers</b>                                     | <b>Estimated numbers per severity</b> |             |                 |               |
|   |  |  | <b>Non-recovery</b>                   | <b>Mild</b> | <b>Moderate</b> | <b>Severe</b> |
|   | Domestic fowl  | 3000   | 0                                     | 0           | 0               | 3000          |
|   |  |  |                                       |             |                 |               |
|   |  |  |                                       |             |                 |               |
| <p>What will happen to the animals kept alive at the end of the procedure?</p>  | <b>Estimated number to be reused</b>   | <b>Estimated number to be returned to habitat/husbandry system</b> | <b>Estimated number to be rehomed</b> |             |                 |               |
|   |  |  | 3000                                  |             |                 |               |
| <p>Please provide reasons for the planned fate of the animals after the procedure.</p>  | Birds will be returned to their commercial flock   |  |                                       |             |                 |               |
| <b>Application of the Three Rs</b>  |  |  |                                       |             |                 |               |
| <p><b>1. Replacement</b><br/>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.</p>   | <p>In order to be able to study the immune mechanisms of chickens during vaccination against rubella, blood sampling is required. Sampling of chickens is necessary, as the physiology and immune system of chickens is "unique" to this species, and information from other animal species is not directly translatable to chickens. The blood samples will be examined using a number of laboratory methods. Among other things, a method where we can study how the hens' white blood cells phagocytose ("eat up") rubella bacteria will be used. This method has been developed by the research group in a previous study and can be seen as a model to partially replace the use of animals in infection studies because it reflects the individual's ability to defend against rubella bacteria.</p> |  |                                       |             |                 |               |
| <p><b>2. Reduction</b><br/>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.</p> | <p>The number of animals for sampling is selected to obtain a representative sample material and statistically reliable results.</p>   |  |                                       |             |                 |               |

|  |  |
|--|--|
| <p><b>3. Refinement</b><br/>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.</p> | <p>The sampling will be performed by a veterinarian with experience in blood sampling of poultry. Other handling will be performed by people with knowledge, experience and experience of poultry. All in all, this reduces the time for restraint and the risk of any negative experiences during sampling.</p> |
| <p>Explain the choice of species and the related life stages.</p>  | <p>Studies in chickens are necessary as the physiology and immune system of the chickens are "unique" to the species and information from other species is not directly translatable to chickens.</p>  |

Some information in this example is of good quality (e.g. objectives, benefits and Replacement).

However, overall the quality is considered poor because:

- 90 months has been stated for the duration. Projects can be a maximum of 60 months
- Predicted harms:
  - Animals will undergo a single blood sampling procedure, however, their experience is categorised as severe, which is inaccurate
- It is stated that chickens will be rehomed but instead they will be returned to habitat
- No reasons given for planned fate of animals
- There is limited information on the implementation of the 3Rs:
  - No information has been provided on "practices that will be used throughout the project to minimise the number of animals used"
  - No information is provided on "mechanisms to take up emerging refinement techniques during the lifetime of the project"
- The choice of species has been justified but the life stage has not



### 3. REGULATORY TESTING

#### Example 5 (good quality)

|   |  |
|---|--|
| <b>Title of the project</b>   | Regulatory project: Conduct of the animal ( <i>in-vivo</i> ) phase of the influenza virus attenuation assay in ferrets   |
| <b>Duration of project</b><br>(in months)   | 12 months  |
| <b>Keywords</b>   | Respiratory flu; vaccine inoculation; immunisation; inactivated virus; batch potency testing   |
| <b>Purpose of project</b><br>(multiple choices possible)  | Regulatory use and routine production – quality control (including batch safety and potency testing)   |
| <b>Objectives and predicted benefits of the project</b>   |  |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | Influenza, commonly known as the flu in humans, is an infectious disease caused by the influenza virus. Common symptoms include a high temperature, aches, runny nose, sore throat, coughing and headaches. However, this is not to be confused with the common cold, as influenza is a more severe disease. Influenza is particularly dangerous to more vulnerable people in society (e.g. children, pregnant women and the elderly), and can potentially develop into pneumonia, which is a life threatening condition. Since influenza virus changes all the time, every year health authorities determine which strains are most likely to be encountered in the upcoming year so that vaccine manufacturers can make the new vaccine (i.e. a new vaccine is required every year). Part of the process of creating each new vaccine involves attenuating the virus. This means that the virus is altered so that its inclusion in the vaccine does not cause illness when it is administered to humans, but instead prompts the human body to develop an antibody response to fight that particular strain of the virus, and therefore prevent infection. Each bulk lot of the live, attenuated vaccine that is manufactured must be tested on live animals to ensure it remains attenuated and does not revert to a virulent (infectious) state, so that it does not result in illness when administered to humans. |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). | <p>The approved human medicine being tested in this project is a unique influenza vaccine that contains live, but attenuated, influenza virus. The vaccine is especially effective in young children, which is helpful to control influenza infections. This will not only benefit the children who receive the vaccine, but also the general population through "herd immunity". Herd immunity occurs when a significant portion of a population is vaccinated, resulting in a higher level of protection for those who remain unvaccinated (certain individuals may not be able to get vaccinated due to allergies etc.). Therefore, this project will result in the availability of a vaccine against the influenza virus, in order to protect human health.</p> <p>This particular vaccine is administered through the nose rather than by injection, so that the infection is stopped at the point of entry (as infection usually occurs by breathing in the virus) and prevents even the</p>   |

|  |   |                                |  |             |                                       |               |
|--|---|--------------------------------|--|-------------|---------------------------------------|---------------|
|  | earliest onset of disease. The other benefit of a nasal vaccine is that avoids pain associated with injection, and this is especially appealing for vaccinating children.   |                                |  |             |                                       |               |
| <b>Predicted harms</b>   |   |                                |  |             |                                       |               |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.                              | Ferrets will undergo a single multi-step procedure consisting of a number of interventions. They will have a blood sample collected under anaesthesia, and their body temperatures will be measured rectally. Ferrets will also receive antibiotics via injection. A few drops of either the vaccine or influenza virus will be introduced into each nostril while the ferrets are under general anaesthesia. The maximum duration of this multi-step procedure is 28 days.   |                                |  |             |                                       |               |
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects? | The anaesthetic might cause mild discomfort but no other adverse effects are expected. The ferrets' body temperatures will be monitored rectally, which may result in some temporary discomfort to the animals. Antibiotic injections may cause temporary pain due to the introduction of a needle. A few drops of either the vaccine or influenza virus will be introduced into each nostril while the ferrets are under general anaesthesia. The administration of the vaccine will not result in any pain or distress, as the animals will be unconscious. However, the ferrets that receive the live (but non-attenuated) virus may develop mild symptoms of influenza, such as increased body temperature and sneezing for up to 3 days. |                                |  |             |                                       |               |
| What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?                        | <b>Species</b>  | <b>Estimated total numbers</b> | <b>Estimated numbers per severity</b>                              |             |                                       |               |
|  |   |                                | <b>Non-recovery</b>  | <b>Mild</b> | <b>Moderate</b>                       | <b>Severe</b> |
|  | Ferrets   | 1200                           | 0  | 1200        | 0                                     | 0             |
|  |   |                                |  |             |                                       |               |
|  |   |                                |  |             |                                       |               |
| What will happen to the animals kept alive at the end of the procedure?  | <b>Estimated number to be reused</b>  |                                | <b>Estimated number to be returned to habitat/husbandry system</b> |             | <b>Estimated number to be rehomed</b> |               |
| Please provide reasons for the planned fate of the animals after the procedure.  | All of the animals will be humanely euthanised at the end of the study, and blood and tissues collected for further analysis.   |                                |  |             |                                       |               |
| <b>Application of the Three Rs</b>   |   |                                |  |             |                                       |               |
| <b>1. Replacement</b><br>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.                                    | Ferrets are used, as they are susceptible to human influenza strains and are an appropriate species for assessing the immune response to influenza vaccines, which is necessary to conduct regulatory projects of this nature. It is not possible to achieve the objectives of this project without the use of live animals, as their use is required for regulatory purposes in order to confirm the safety of the vaccine. The studies are carried out in accordance with European guidance and laws on the testing of medicinal products for use in humans.  |                                |  |             |                                       |               |

|   |  |
|---|--|
| <p><b>2. Reduction</b><br/>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.</p> | <p>Statistical methods have been used to ensure the minimum numbers of animals are being used in order to achieve the objectives of the project. The numbers chosen are based on European regulatory guidelines for this particular type of test.</p>  |
| <p><b>3. Refinement</b><br/>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.</p>  | <p>General anaesthesia will be used when necessary to minimise any stress or discomfort to the ferrets during these procedures. Positive reinforcement techniques (i.e. provision of treats) will be applied after each procedure to reward the ferrets. The animals will be observed twice a day, and if any animal becomes ill beyond the expected and acceptable mild adverse effects, a veterinarian will be consulted and appropriate treatment instigated.</p> |
| <p>Explain the choice of species and the related life stages.</p>   | <p>Adult ferrets will be used, as this is the species required by law for this type of testing.</p>  |

**This is considered a good quality NTS because:**

- **Language is clear concise and understandable to a layperson**
- **Scientific terms are defined**
- **It is anonymous**
- **Clear informative keywords specific to this research are provided**
- **The tertiary project purpose has been included as a keyword**
- **Objectives are clearly described**
- **Benefits are clearly described but not overstated**
- **It is clear that animal use is required in accordance with regulatory requirements**

- **All of the potential harms and their expected duration are listed (i.e. rather than referring to regulatory guidelines with which a lay audience would not be familiar)**
- **Information on implementation on each of the 3Rs is provided, including:**
  - **Clear information on why replacement is not possible to achieve the scientific objectives**
  - **Information on reduction and refinement measures (although relatively limited for regulatory testing of this nature)**
- **Information is provided to justify the species and life stage used**

DRAFT

**Example 6 (poor quality)**

|   |  |
|---|--|
| <b>Title of the project</b>   | Biocompatibility assessment of medical devices – orthopaedic screws  |
| <b>Duration of project</b><br>(in months)   | 48 months  |
| <b>Keywords</b>   | Irritation; sensitization; rabbits; mice   |
| <b>Purpose of project</b><br>(multiple choices possible)  | Regulatory use and routine production – toxicity and other safety testing including pharmacology   |
| <b>Objectives and predicted benefits of the project</b>   |  |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | <p>These tests are required by law or in manufacturing (toxicity and other safety studies, including pharmacology) and involve testing for skin irritation and skin sensitisation.</p> <p>The purpose of the study is to determine whether medical devices (orthopaedic screws) show irritant response (intra-dermal sensitivity test) and sensitisation (LLNA). Medical devices (orthopaedic screws) allow bone stability to bring the bone fragments into the desired position and in the shortest possible time. The use of medical devices eliminates to a minimum the need for additional immobilisation, such as a plaster cast, thus improving the patient’s standard of living. Medical devices can be used in upper and lower limb surgery, and in the shoulder and pelvic girdle. The study will be performed to determine the safe use of medical devices dedicated to treating diseases or dysfunctions in humans and animals.</p> |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). | <p>The experiment will be performed to determine the safe use of medical devices dedicated to treating diseases or dysfunctions in humans and animals. According to Table A1 of ISO 10993-1: “Biological evaluation of medical devices” (which is a harmonised standard) it is necessary to assess irritation and sensitisation. The tests shall be carried out in accordance with ISO 10993 Part 10.</p> <p>If the desired test results are obtained, orthopaedic screws may be used to support the treatment of humans and animals.</p>  |
| <b>Predicted harms</b>  |  |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.   | Rabbits will undergo a surgery to implant orthopaedic screws in the shoulder under general anaesthesia.  |

|  |   |  |                                       |                                       |                 |               |
|--|---|--|---------------------------------------|---------------------------------------|-----------------|---------------|
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?   | N/A   |  |                                       |                                       |                 |               |
| What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?  | <b>Species</b>  | <b>Estimated total numbers</b>                                     | <b>Estimated numbers per severity</b> |                                       |                 |               |
|  |   |  | <b>Non-recovery</b>                   | <b>Mild</b>                           | <b>Moderate</b> | <b>Severe</b> |
|  | European Rabbit   | 18   | 0                                     | 18                                    | 0               | 0             |
|  | Mouse   | 90   | 0                                     | 90                                    | 0               | 0             |
|  |   |  |                                       |                                       |                 |               |
| What will happen to the animals kept alive at the end of the procedure?  | <b>Estimated number to be reused</b>  | <b>Estimated number to be returned to habitat/husbandry system</b> |                                       | <b>Estimated number to be rehomed</b> |                 |               |
| Please provide reasons for the planned fate of the animals after the procedure.  | Mice will be euthanised at the end of the study for the collection of tissues and downstream analysis.  |  |                                       |                                       |                 |               |
| <b>Application of the Three Rs</b>   |   |  |                                       |                                       |                 |               |
| <b>1. Replacement</b><br>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.  | In the case of biocompatibility studies on safety of the use of a medical device, it is currently not possible to replace the experimental procedure (using live vertebrate animals) by another testing method. |  |                                       |                                       |                 |               |
| <b>2. Reduction</b><br>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, | The total number of animals used in the experiment has been kept to a minimum.  |  |                                       |                                       |                 |               |

|  |  |
|--|--|
| sharing of tissue and reuse.   |  |
| <b>3. Refinement</b><br>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project. | All activities will be performed in accordance with ISO 10993 Part 10: "Skin irritation and sensitisation testing".  |
| Explain the choice of species and the related life stages.   | In accordance with the guidelines of ISO 10993 Part 10, the evaluation of the irritating effect of the medical device shall be carried out on the rabbits, and the LLNA test on domestic mice. |

**This is considered a poor quality NTS because:**

- **The title is vague**
- **Keywords are not informative**
- **Keywords include species (rabbits and mice), which should not be included as keywords as they are captured elsewhere**
- **Third level purpose should be included as a keyword**
- **Acronyms are not defined**
- **Predicted harms:**
  - **Harms have been included for rabbits only. However, mice will also be used and their experience has not been described. Duration of procedure not included**
  - **"N/A" has been included in response to the adverse effects question – this is not appropriate as it is stated that rabbits will undergo surgery and therefore adverse effects such as post-operative should be expected**
- **The severity listed for both rabbits and mice is mild. This is incorrect for rabbits, as they will undergo an invasive surgical procedure under anaesthesia. It is unclear whether this classification is appropriate for mice, as the harms have not been described**
- **Reasons for the planned fate of the animals has been included for mice only – it is unclear what will happen to the rabbits at the end of the study**
- **There is limited information on the implementation of the 3Rs:**
  - **A number of well-validated *in vitro* (non-animal) tests are available to assess skin irritation and sensitisation. These have not been described in the Replacement section and therefore the use of live animals has not been adequately justified**
  - **It should be stated that statistical calculations were performed to determine the appropriate number of animals for use**

- **No information has been provided on “practices that will be used throughout the project to minimise the number of animals used”**
- **No information is provided on “mechanisms to take up emerging refinement techniques during the lifetime of the project”**
- **There is an over-reliance on referring to the regulatory guidelines. The lay person would not be familiar with this guidelines – more simple information is required to justify the choice of species and life stage**

DRAFT



#### 4. EDUCATION AND TRAINING

##### Example 7 (good quality)

|   |  |
|---|--|
| <b>Title of the project</b>   | Teaching and assessing competency in clinical skills involving cattle relevant to veterinary degree programmes   |
| <b>Duration of project</b><br>(in months)   | 12 months  |
| <b>Keywords</b>   | Veterinary trainees' education; clinical skills practice; reproductive technology; cattle management;  |
| <b>Purpose of project</b><br>(multiple choices possible)  | Higher education<br>Training for the acquisition, maintenance, or improvement of vocational skills   |
| <b>Objectives and predicted benefits of the project</b>   |  |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | The overall purpose and objective of this project is to give both veterinary students and nurses the best education possible, and make sure they become professionally competent individuals who can provide a good standard of care to animals when they qualify. The teaching and assessment of basic animal husbandry procedures, as well as practical clinical competencies, is an essential component of veterinary education and is a requirement of the national competent authority for the veterinary profession and international accrediting organisations. The aim of this project is to train undergraduate veterinary medicine and veterinary nursing students in standard and specialist veterinary practices in cattle. Training using live animals is essential in order for students to gain competence in performing procedures that they will use throughout their veterinary careers. |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). | The ability to perform techniques such as taking blood samples and administering veterinary therapeutics to cattle is mandatory for any qualified veterinarian or nurse, in order to diagnose and treat disease and participate in national disease eradication programmes. A subset of students who wish to develop specialist skills in cattle reproduction will also learn these important skills as part of this project, which they will eventually use in practice. Therefore, the benefit that will arise from this project is that it will improve the husbandry and clinical competencies of both veterinary medicine and veterinary nursing students so that they are better equipped for their careers upon qualification.  |
| <b>Predicted harms</b>  |  |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and   | Over the course of this training project, animals will be subjected to one of three procedures, depending on the skills being trained. Adult cattle will undergo brief restraint, rectal and mammary examinations, blood and urine sampling, and various injection and oral administration techniques. Calves will undergo restraint, placement of a tube through  |

|  |   |  |                                       |                                       |                 |               |
|--|---|--|---------------------------------------|---------------------------------------|-----------------|---------------|
| duration of these procedures.  | the mouth to the stomach for feeding, and a small number may receive a nerve block (local anaesthetic) by injection. A separate group of adult cattle will be used for specialist training in reproductive interventions such as induction of oestrus synchronisation (i.e. so that animals come into oestrus/heat at the same time) and embryo recovery. Typically, the procedures will be performed once per week for 6 weeks or 12 weeks. However, the reproduction skills training procedure will only be performed twice per year. The majority of the interventions are short-lived ( $\leq 30$ minutes), though embryo recovery may take up to 1 hour. |  |                                       |                                       |                 |               |
| What are the expected impacts/adverse effects on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects? | Animals receiving injections or undergoing blood sampling may experience slight pain or discomfort at the site of needle insertion. Cattle may also experience discomfort during oral dosing, feeding tube placement, and rectal examination, as well as mild stress due to repeated restraint. Female cattle undergoing oestrus synchronisation and embryo recovery may also experience brief pain or discomfort when receiving injections, or due to insertion of a catheter into the cervix to collect embryos. In all instances, these effects are transient and no long-term adverse effects are expected.   |  |                                       |                                       |                 |               |
| What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?                        | <b>Species</b>  | <b>Estimated total numbers</b>                                     | <b>Estimated numbers per severity</b> |                                       |                 |               |
|  |   |  | <b>Non-recovery</b>                   | <b>Mild</b>                           | <b>Moderate</b> | <b>Severe</b> |
|  | Cattle  | 160  | 0                                     | 160                                   | 0               | 0             |
|  |   |  |                                       |                                       |                 |               |
|  |   |  |                                       |                                       |                 |               |
| What will happen to the animals kept alive at the end of the procedure?  | <b>Estimated number to be reused</b>  | <b>Estimated number to be returned to habitat/husbandry system</b> |                                       | <b>Estimated number to be rehomed</b> |                 |               |
|  |   | 160  |                                       |                                       |                 |               |
| Please provide reasons for the planned fate of the animals after the procedure.  | After the end of the teaching period, all animals will return to the herd, where they will live normal lives as farm animals.   |  |                                       |                                       |                 |               |
| <b>Application of the Three Rs</b>   |   |  |                                       |                                       |                 |               |
| <b>1. Replacement</b><br>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.                                    | It is necessary for students to become competent in common veterinary procedures in live animals in order to eventually perform them on animals under their care as qualified veterinarians and veterinary nurses. However, students will attend lectures, watch videos and practice techniques on non-animal models, dummy animals and cadavers prior to using live animals, in order to develop as much competency as possible. Nevertheless, progression to using live animals is necessary for students to attain full competence.  |  |                                       |                                       |                 |               |
| <b>2. Reduction</b><br>Explain how the numbers of animals for this project   | The number of animals selected for use is based on the number of veterinary and veterinary nursing students enrolled in the degree programmes at any one time. The minimum number of animals that will  |  |                                       |                                       |                 |               |

|   |  |
|---|--|
| <p>were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.</p> | <p>allow students to demonstrate successful competence in performing procedures without jeopardising the animals' welfare will be used. Where possible multiple mild interventions will be performed in the same animals to reduce the total number of cattle required.</p>  |
| <p><b>3. Refinement</b><br/>Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project.</p>  | <p>Interventions will be carried out under close supervision of a trained and experienced veterinarian who will provide veterinary assistance and advice should any animal welfare issues arise. For some procedures, epidural anaesthesia or sedation will be used as appropriate in order to avoid the animal feeling any discomfort. Animals will be monitored closely for any signs of adverse effects post interventions.</p> <p>Veterinary educators will perform regular literature reviews and consult 3Rs resources in order to keep up-to-date with emerging refinement opportunities that could be applied to this project.</p> |
| <p>Explain the choice of species and the related life stages.</p>   | <p>Cattle have been chosen for use, as they are the target species in which the students need to train. Calves and adult cattle will be used, as training is required in both of these life stages.</p>  |

**This is considered a good quality NTS because:**

- **Language is clear concise and understandable to a layperson**
- **It is anonymous**
- **Clear informative keywords specific to the project are provided**
- **Objectives are clearly described**
- **Benefits are clearly described but not overstated**
- **All of the potential harms and their expected duration(s) are listed**
- **Information on implementation on each of the 3Rs is provided, including:**
  - **Clear information on why replacement is not possible to achieve the objectives in terms of training and education**
  - **Details are provided on the Reduction measures being applied**
  - **Comprehensive information on Refinement measures is included**
- **Information is provided to justify the species and life stage used**

**Example 8 (poor quality)**

|   |   |
|---|---|
| <b>Title of the project</b>   | Development of medical skills of medical and veterinary medical students of University of Europa, and junior doctors, and doctors from University Hospital Europa for the improvement of the quality of their work  |
| <b>Duration of project</b><br>(in months)   | 60  |
| Key Words (maximum of 5) <sup>1</sup>   | Surgery ; education ; training; pigs  |
| <b>Purpose of project<sup>2</sup></b><br>(multiple choices possible)  | Higher education<br>Training for the acquisition, maintenance, or improvement of vocational skills  |
| <b>Objectives and predicted benefits of the project</b>   |   |
| Describe the objectives of the project (for example, addressing certain scientific unknowns, or scientific or clinical needs).  | Provision of a theoretical and practical course for medical and veterinary medical personnel, during which the participants learn correct and safe actions during medical and veterinary medical manipulations and surgical operations.   |
| What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the project. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). | Trainees that have completed the courses will have obtained irreplaceable skills and will have developed their current knowledge of, and skills pertaining to, complex surgical techniques and manipulations. Such knowledge and skills will significantly decrease the potential risk of a doctor or veterinary surgeon making a mistake, and will improve medical and veterinary medical care in general. |
| <b>Predicted harms</b>  |   |
| In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures.   | Animals will be anaesthetized throughout.   |
| What are the expected impacts/adverse effects   | N/A   |

|   |   |                                |  |             |                                       |               |
|---|---|--------------------------------|--|-------------|---------------------------------------|---------------|
| on the animals, for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?  |   |                                |  |             |                                       |               |
| What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?   | <b>Species<sup>4</sup></b>  | <b>Estimated total numbers</b> | <b>Estimated numbers per severity</b>                              |             |                                       |               |
|   |   |                                | <b>Non-recovery</b>  | <b>Mild</b> | <b>Moderate</b>                       | <b>Severe</b> |
|   |   |                                |  | 150         |                                       |               |
|   |   |                                |  |             |                                       |               |
|   |   |                                |  |             |                                       |               |
| What will happen to the animals kept alive at the end of the procedure? <sup>5, 6</sup>   | <b>Estimated number to be reused</b>  |                                | <b>Estimated number to be returned to habitat/husbandry system</b> |             | <b>Estimated number to be rehomed</b> |               |
| Please provide reasons for the planned fate of the animals after the procedure.   | Animals will not be awakened from the anaesthetic they are placed under. At the end of the anaesthetic pigs will be euthanised.   |                                |  |             |                                       |               |
| <b>Application of the Three Rs</b>  |   |                                |  |             |                                       |               |
| <b>1. Replacement</b><br>State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project.   | The participants in the courses, prior to starting their work with the study animals, will develop their skills using pre-prepared computer simulation devices. The study animals will be used to perform only those procedures and manipulations that cannot be learned using tissue cultures. |                                |  |             |                                       |               |
| <b>2. Reduction</b><br>Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used to design studies. Where applicable, describe practices that will be used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. | The number of study animals has been calculated to make sure that as few animals as possible are exposed to the procedures.   |                                |  |             |                                       |               |
| <b>3. Refinement</b>  | Animals will be under anaesthesia throughout the procedure and euthanised without recovery.   |                                |  |             |                                       |               |

|  |          |                            |                         |              |
|--|----------|----------------------------|-------------------------|--------------|
| Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project. |          |                            |                         |              |
| Explain the choice of species and the related life stages.   | N/A      |                            |                         |              |
| <b>Project selected for Retrospective Assessment<sup>7</sup></b>   | Deadline | Contains severe procedures | Uses non-human primates | Other reason |

**This is considered a poor quality NTS because:**

- **The title is vague**
- **The title is not anonymous and confidentiality is not adequately protected - the university and the hospital these students and doctors are associated with are named.**
- **Keywords are not informative – they are too general and vague**
- **Keywords include species (pigs). Species is captured elsewhere in the NTS therefore does not need be included as a keyword.**
- **The objectives and potential benefits sections both lack adequate detail to support the merits of the project. In both cases the information provided is scant and vague. There is no description of the specific techniques/skills that participants will be trained in, or of the particular medical/surgical fields these doctors are working in. Hence it is not possible to determine the true necessity of the training proposed.**
- **Predicted harms:**
  - **The procedure(s) that these pigs will undergo has/have not been described so it is impossible for the reader to get an accurate sense of the actual experience of the animals used under this authorisation. Furthermore, there is no information provided on the number or duration of the procedure(s) to be performed.**
- **“N/A” has been included in response to the adverse effects question – this is not appropriate. Although the detail elsewhere in the NTS is scant, it appears that these animals will be placed under terminal anaesthesia for a period of time, and then killed at the end of the anaesthetic. This being the case, they may experience stress during anaesthetic induction. Again, no duration for expected effects has been provided. The severity listed for all the pigs to be used is mild. Given that the detail provided on the predicted harms and adverse effects sections is entirely inadequate, it is not possible to state with confidence what the most appropriate prospective severity/severities is/are. However, based on the detail provided elsewhere, it appears that these animals will be**

placed under terminal anaesthesia for a period of time, and then killed at the end of the anaesthetic. This being the case, the severity for these animals should be non-recovery.

- No reasons are provided for the planned fate of these animals.
- There is limited information on the implementation of the 3Rs:
  - Replacement – the use of a tiered approach to this training is not adequately described. There is no reference to theoretical learning, watching videos, direct observation of experienced tutors/colleagues, the use of dummy animals and cadavers. Therefore the absolute requirement to use live animals has not been sufficiently justified
  - Reduction - no information has been provided on “practices that will be used throughout the project to minimise the number of animals used” e.g. sharing of animals by a number of students/doctors etc, and the origin of the animals (e.g., surplus stock animals, animals from completed studies that have not yet been euthanised)
  - Refinement - no information is provided on “mechanisms to take up emerging refinement techniques during the lifetime of the project”
- No information has been provided in support of the choice of pigs as a species to be used for this training, nor on the life-stage of pig to be used, and why this species/life-stage are the most suitable to achieve the objectives of the study.