Home Office

Guidance

Notes for Project Licence Applications

September 2022

Version 1

Will you be producing genetically altered or surgically prepared animals/animal products using standardised protocol frameworks as a service to others?
This includes projects to create, breed, maintain and supply genetically altered animals to researchers within the establishment, projects taking blood and other tissues for researchers and other clients within and/or external to the establishment.

Yes/No

Will you be manufacturing vaccines and medicines for medical or veterinary use?

Yes/No

Do you need to transfer animals from a project that's due to expire?

Yes/No

Project licence number

PPL Number

Expiry date
For example, 13 06 2019

01/01/2025

Action plan

Objective 1

Objective title

Describe objectives to achieve the overall aim. You may wish to describe these objectives as questions (see examples below). Objectives should be realistically achievable based on available funding, staffing and other resources. You may not be able to identify all objectives at the time of application. Additional objectives can be added as amendments if they fit within the overall aim.

Usually, general terms like "cancer", "nervous system", "receptors" or "pathways" are too broad without specific explanation of what you are aiming to do e.g., Which types

of cancer? What aspects of disease? What elements of the nervous system, with what effects?

Try to ensure your objectives reflect the outcomes you want to achieve, not the methods you will be using to achieve the outcomes. For example, creating a new line of genetically altered mice is a method of achieving a scientific objective rather than the scientific outcome desired.

Basic research example: Does mis-expression of candidate genes affect the stem cell compartment and/or alter tumour susceptibility in animals? How would anti-cancer drugs alter the Myc and other (p53/Ras) oncogenic systems?

Objective 2

Objective title

Translational research example: Determine the pathogenesis of the infectious organisms in pregnant and neonatal pigs

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How do each of these objectives relate to each other and help you to achieve your aim?

Outline any interdependencies, stop: go points, and milestones. Include any key in vitro, ex vivo or in silico work, clinical findings, or results from epidemiological studies carried out under other projects that will enable you to achieve your objectives. Consider including images (.jpg and .png files) of annotated flow charts and decision trees in your action plan to illustrate how objectives relate to each other.

We need a high- level overview of the scientific strategy to achieve the aim and each objective, including in vivo, ex vivo or non-animal studies that contribute to decision making of the in vivo work and/or realising the benefits. For example, in a drug discovery programme, initial identification of molecules that interact with the target will normally be done in vitro or from a literature search.

This section links the objectives to the protocols and explains how objectives will be achieved. There should be an outline of the stages of the programme of work and indication, using the protocol numbers, how each protocol will be used to achieve each objective.

Consider using an annotated flow chart, process map or decision tree with a short supporting narrative to summarise your strategic approach. This is often the clearest way of representing how the different objectives relate to each other and how the protocols will contribute to the objectives. Your process maps/decision trees can be uploaded and inserted as images (.jpeg or .png files). For each objective, indicate briefly what inputs (e.g., information, validated models & reagents etc.) are necessary and what outputs (for example data, models or products) are expected. Where a programme of work has several sequential stages, explain the criteria for progressing to the next stage. Do not include detailed descriptions of the procedures or models at this stage.

An example of a process map is provided at the end of this section that illustrates the proposed work plan for a project investigating whether candidate genes have a role in energy and/or metabolic disorders. Note that the process map is based around the objectives the work is aiming to answer. It also shows the sequence of studies, work carried out prior to, and after this project, and the protocols used.

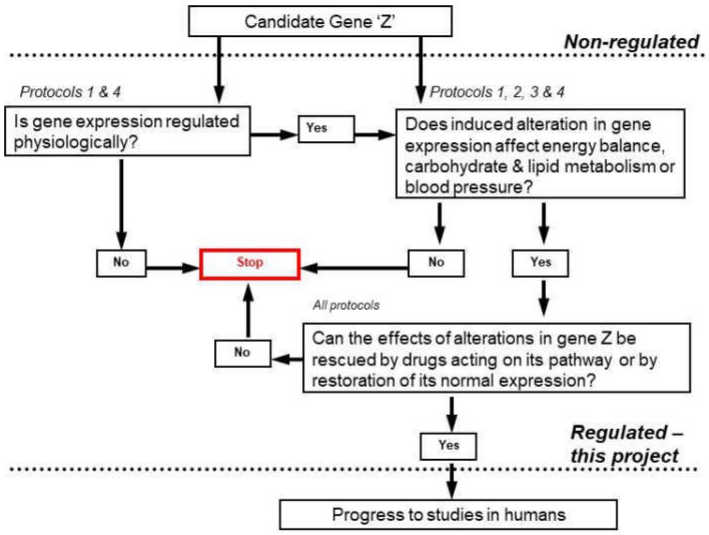
Whether or not you use a process map, add here a brief explanatory narrative to explain how decisions will be taken to determine how to navigate through the project. Show how and where the work of your project fits in with in silico, in vitro and ex vivo procedures and with work carried out under other projects or elsewhere.

Explain succinctly and in turn how each objective will be addressed and achieved. To do this, consider what output (i.e., data to be acquired, results or products to be generated) is needed to achieve the objective or the aim of the project as a whole.

Example of a simple GA mouse research project:

'To determine whether gene x plays a role in the development of diabetes, we will create mice that either don't express the gene or overexpress the gene (which relates to clinical findings). We will use standard protocols 1-6 (superovulation, generation of founders, vasectomy, embryo recipient, breeding & maintenance mild and moderate). These mice will be used in a diabetes induction protocol (protocol 7) and the degree of development of diabetic signs will be measured.'

Decision tree example: Investigating whether candidate genes have a role in energy and/or metabolic disorders



Where relevant, how will you seek to use or develop non-animal alternatives for all or part of your work?

Example: During the course of the project, we will continue to look for non-animal alternatives for any aspect of our work and we will use tools such as SyRF, the free

online platform for researchers, to perform a systematic review and meta-analysis of animal studies.

Will you be producing data primarily for regulatory authorities that use standardised protocol frameworks?

Yes/No (Typically ‘no’ for purpose a, b, and non-regulatory purpose c)

Will you be undertaking non-regulatory testing or screening as a service to others?

Yes/No (Typically ‘no’ for purpose a, b and non-regulatory purpose c)

Will you be producing genetically altered or surgically prepared animals/animal products using standardised protocol frameworks?
This includes projects to create, breed, maintain and supply genetically altered animals to researchers within the establishment, projects taking blood and other tissues for researchers and other clients within and/or external to the establishment.

Yes/No

Will you be manufacturing vaccines and medicines for medical or veterinary use?

Yes/No (Typically ‘no’ for purpose a, b and non-regulatory purpose c)

General principles

Unnecessary duplication of work must be avoided. Under what circumstances would you knowingly duplicate work?

We need an explanation of steps taken to ensure testing you are doing will not duplicate tests already done or data already available. Replicating research findings of others might well be a legitimate part of a research programme.

Example: The genetically altered animals will be mice. Where suitable lines already exist, animals will be obtained from the relevant supplier. Otherwise, we will make the required lines ourselves (including conditional knockouts).

Example: Experimental reproducibility is an extremely important part of scientific investigation. Many experimental procedures involve technically demanding protocols, often using experimental reagents. It is essential that such experiments are repeated to provide confidence. Irrespective of power calculations, it is critical that experimental reproducibility is ensured.

Will all of your protocols or experiments use animals of both sexes?

Yes/No

_____

Why will you use animals of a single sex in some protocols or experiments?

We require a robust explanation as to why animals of both sexes cannot be used. It is not enough to simply answer male mice fight so we will have to keep them singly housed or female mice result in high variability due to oestrus cycles. You need to explain why you cannot factor in sex differences (if any) into your experimental design. There are obvious exceptions e.g., studies on prostate cancer.

Protocols

General constraints

Please note, constraints on procedures involving anaesthesia, surgery, substance administration and withdrawal of fluids apply to all protocols.

Anaesthesia

Induction and maintenance of general or local anaesthesia, sedation, or analgesia to mitigate the pain, suffering or distress associated with the performance of other regulated procedures is indicated using the following codes in protocols:

- AA no anaesthesia
- ABL local anaesthesia
- AB general anaesthesia with recovery
- AC non-recovery general anaesthesia
- AD under neuromuscular blockade

General anaesthesia

If authorised in this licence and unless otherwise specified, all animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Uncommonly animals that fail to do so or exhibit signs of pain, distress or of significant ill health should be humanely killed unless a programme of enhanced monitoring and care is instituted until the animal fully recovers.

Surgery

If authorised in this licence and unless otherwise specified:

- Surgical procedures should be carried out aseptically, to at least the published Home Office minimum;
- In the uncommon event of post-operative complications, animals will be humanely killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than

minor interventions. Minimally inflamed wounds without obvious infection may be re-closed on one occasion within 48 hours of the initial surgery. In the event of recurrence, NVS advice will be followed;

- Peri and post-operative analgesia will be provided; agents will be administered as agreed in advance with the NVS;
- All animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Uncommonly animals that fail to do so or exhibit signs of pain, distress or of significant ill health will be humanely killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers;
- Any animal not fully recovered from the surgical procedure within 24 hrs (eating, drinking and return to normal behaviour) should be humanely killed.

Administration of substances and withdrawal of fluids

If authorised in this licence and unless otherwise specified, administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes, and frequencies that of themselves will result in no more than transient discomfort and no lasting harm using published guidelines on minimal severity.

Protocol 1

Title

The title should reflect the purpose for which the protocol will be used and be reasonably short e.g., A murine model of inflammatory bowel disease (IBD)

Protocol 1: Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

By ‘regulatory guidelines’ we mean guidelines Regulators have published such as the OECD guidelines.

Provide a brief summary of the purpose of the protocol.

Example: Induction of colitis for the testing of novel IBD therapies

Example: To induce subcutaneous and metastatic tumours and study response to treatments

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Given the controls and limitations in place, what is the highest severity that an animal could experience in this protocol?

Moderate

What proportion of animals will experience this severity?

The severity category of the protocol is essentially a label that conveniently captures the maximum level of harm likely to be experienced (the worst case likely harms for each protocol – don't include unexpected events). See section 5.12 of The Home Office Guidance.

Licences with protocols classified as severe will be subject to Retrospective Assessment. See section 5.17 of the Home Office Guidance.

Do not set the severity category until you have described the likely adverse effects of the individual procedures and their combined results, the control measures and humane endpoints.

Refer to section 5.7.3 and Appendix G of the Home Office Guidance for more information about severity classification.

The final decision on the severity category for any protocol will be taken by ASRU. If this differs from the classification you have proposed, the application will be returned to draft on ASPeL for you to update and resubmit.

The prospective severity must be determined using the principles at Guidance section 5.12 and Appendix G, and 'Severity classification of genetically altered animals under the Animals (Scientific Procedures) Act 1986'

The Inspector will confirm that this is correct as part of their assessment.

Example: 'Approximately 75% of animals are likely to experience moderate levels of severity. The remaining 25% of animals are likely to experience mild severity'.

Why are you proposing this severity category?

This section should include a description of the maximum level of harm likely to be experienced.

Example: We are proposing this severity category because 75% of animals will undergo surgery to occlude one ureter to induce inflammation in the associated kidney, repeated single housing in metabolic cages (once weekly for up to 12 weeks) and repeated blood sampling and dosing of substances. The remaining 25% of animals are likely to experience mild severity because they will not undergo the surgical preparation procedure and we expect the cumulative effect of the other procedures to be mild.

Locations where this protocol can be carried out

Select all that apply.

- Establishment 1

Which of your objectives will this protocol address?

Select all that apply.

- Translational research example: Determine the pathogenesis of the infectious organisms in pregnant and neonatal pigs

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Protocol 1: Animals used in this protocol

Mice

Which life stages will be used during this protocol?
Select all that apply

- Juvenile
- Adult

Will any animals coming on to this protocol be classed as 'continued use'?
'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes/No

How did these animals start their use?
Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Continued use is an administrative way of specifying use over more than one project licence protocol within the same or different project licence(s). The regulated procedures authorised sequentially by the two (or, rarely, more) protocols must be essential to achieve the intended particular purpose. See Advice Note on Use, keeping alive and re-use.

For practical reasons, production of an animal model, e.g., breeding of genetically altered animals or surgical preparation may occur under a separate protocol from the subsequent experimental procedures applied to that model.

Transfer of animals between protocols for continued use in the same, or different, project licences must be authorised in the relevant parts of both protocols. However, the import (from outside the UK) of genetically altered schedule 2 species does not require specific authorisation as continued use as they have not been 'used' under the Act.

Example: Continued use of genetically altered mice: Mice for use in this protocol may be obtained from Protocol X of this project (Breeding and maintenance of genetically altered animals) or other projects with authority to breed and maintain genetically altered animals of that type and to provide them for use on other projects.

Example: Continued use of surgically prepared animals: Rats previously surgically prepared with one or more vascular cannulae at another licensed establishment

under the authority of a Project Licence which permits transfer for continued use may be used in this protocol.

Will you be re-using animals on to this protocol?
'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

Yes/No

Describe any procedure that may have been applied to these animals, and why you are choosing to re-use them.

See the published Advice Note on Use, keeping alive and re-use.

Example: Wild-type genotyped animals from Protocol X (Breeding and maintenance of genetically altered animals) of this licence may be re-used.

Example: Dogs that have been kept alive and maintained under the supervision of the NVS at [place] may be re-used in this protocol, provided that all criteria in section 14 of the Animals (Scientific Procedures) Act and in this project, licence are fulfilled.

What is the maximum number of animals that will be used on this protocol?

Give a realistic estimate of the number of animals to be used in this protocol over the lifespan of the project.

What is the maximum number of uses of this protocol per animal?
For example, if some animals will go through this protocol three more times after their first use, the number of uses will be four. If no animals will go through this protocol more than once, enter '1'.

Where there is re-use on the same protocol the number will be greater than one.

Protocol 1: Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

Which general types or strains will you be using and why?

This series of questions is so that we can assess the justification for using GA animals, especially those that have an adverse phenotype.

- Where GA animals are expected to have a mild phenotype, the description can be general.

- For moderate or severe phenotypes, each genetically altered line or type of line must be specified.

Examples of mild phenotype:

- Immunodeficient mice [when held in suitable housing conditions] as hosts for implanted tumour tissue
- Zebrafish with genetic alterations relating to Y pathways to undertake work to achieve objective A
- Mice with tissue-specific fluorescent genes to allow localisation of [cell types]

Examples of moderate phenotype:

- Mice with alterations in x, y, z genes resulting in an increased risk of liver tumour formation
- Zucker rats as a model of non-insulin-dependent diabetes mellitus
- Mice with recombinant, mutant, knock-out or knock-down (constitutive or conditional) versions of x, y, z genes to study mechanisms of neurodegenerative disease.

Example of severe phenotype:

- Mice with a deletion or other alteration of gene x to identify the role of this gene in heart failure

Do you expect any of these GAAs to show a harmful phenotype with welfare consequences?

Yes/No

Why are each of these harmful phenotypes necessary?

Ensure there is a robust scientific justification for any moderate or severe GAAs.

You should explain:

- Why the harmful phenotypes are necessary to achieve the objectives
- What other GA lines have been considered
- Why GAAs with a lower capacity to experience pain, suffering, distress, or lasting harm be used, such as fish, flies or worms are unsuitable.

How will you minimise the harms associated with these phenotypes?
Ensure that you include any humane endpoints that you will use.

This is for the Harm Benefit Analysis and to assess adequacy of refinement considerations.

Describe the harmful phenotypes and the refinement measures and other controls to minimise harms associated with these phenotypes.

Example: Animals will be killed before 12 weeks of age or at the onset of clinical signs if earlier unless required for experimental use when they will be transferred as continued use to Protocol X.

Protocol 1: Steps

Step 1 (mandatory)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

The rationale for each step must be clear, for example not 'administration of substances' by a number of routes but 'administration of potential therapeutic agents', 'administration of contrast agents', administration of antibiotics' or 'receptor agonists and antagonists', or 'administration of inducing agents'. Each route required should be listed. The exact substance to be used should be specified if it results in significant adverse effects. For example, DSS to induce colitis, CFA as an adjuvant.

Animal models with significantly different adverse effects should be authorised in different protocols, e.g., local benign tumours, malignant cancers, cancers of different organs, etc.

It is acceptable to write: 'unilateral nephrectomy (AB)'; 'Ovariectomy (AB)' etc. This allows for flexibility to use different more refined or more effective techniques without amending the licence. Related surgery, including surgical access and closure of the wounds, is encompassed by these general terms and does not need to be specified.

Be clear about control groups, including sham surgical controls - which steps will they go through? Specify if control substances will be administered.

Anaesthetic codes (AA, ABL, AB, or AC as appropriate to indicate when a procedure is being done under anaesthesia); or AD for procedures undertaken under neuromuscular blockade should be used for each step or sub-step. If administration of analgesia or anaesthesia (e.g., for restraint) is the only regulated procedure associated with an experimental step, this must be included as a separate step. It is helpful to provide details of the typical and maximal number, frequency, and duration of dosing.

For volumes, and for routinely used routes (like those listed in example below), it is acceptable to refer to published guidelines e.g., LASA, Diehl et al (2001) or Turner et al (2011). However, it is still necessary to state frequency of dosing and the total duration of dosing to determine any cumulative effects that may raise the severity classification.

For less commonly used routes, such as intrathecal, intracerebral, intra-articular etc, the maximum dose volumes should be stated, as well as details of frequency and

duration. The same applies to frequency and duration of repeated imaging, foot-shock etc.

Sub-threshold procedures only need to be mentioned as a step if they might cumulatively result in above threshold levels of suffering. For example, repeated short periods of separation from cage-mates, repeated short periods of food restriction or some behavioural tests. These non-regulated procedures can be included as a step.

Setting free to the wild during the course of procedures must be a step (see Advice Note 'Working with wild animals').

Example: Removal of blood samples from a superficial blood vessel to assess haematological parameters (AA/AB/AC)

Example: Confinement/single housing in a metabolism cage (AA) usually up to 24 hours, but occasionally up to a maximum of 5 days.

Example: Immunotherapeutic substances or vehicle control will be administered alone or in combination, continuously or intermittently by one or more of the following routes:

a) in the diet or drinking water (AA)

b) subcutaneous (AA/AB)

c) intraperitoneal (AA/AB)

d) implantation of a slow-release pellet subcutaneously on one occasion (AB)

e) topical application (AA)

Is this step optional?

Yes/No (A minimum of one step should be mandatory)

Do you expect this step to have adverse effects for the animals that are more than mild and transient?
Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

Yes/No (Steps should typically include a description of adverse effects)

What are the likely adverse effects of this step?
State the expected adverse effect, including the likely incidence, and the anticipated degree and duration of suffering.

Note: Common adverse effects, control measures and humane end points for surgery, anaesthesia, substance administration are included as General Constraints in the licence and do not have to be repeated for these steps.

Include the expected clinical signs and incidence. General terminology can be used to describe the incidence of effects: rare (<1%), uncommon (1-5%), common (5-10%), usual, all.

Provide more detail for the procedures that cause the major impacts on animals.

Don't forget to describe the expected adverse effects caused by the substances being administered. For example, chemotherapeutics or transgene inducing agents can result in adverse effects.

Regarding surgery, the standard general constraints text will cover the standard acute effects of surgery. Describe any longer-term consequences resulting from that surgery, e.g., neuropathic pain, stroke, liver failure.

Any adverse effects and humane endpoints relating to a GA phenotype will have been described in the GA animals section above and do not need to be repeated.

Examples:

Unilateral ureteral ligation will cause kidney inflammation in all animals which is not, in our experience, associated with signs of pain and although the affected kidney fails, the animal remains healthy because the other kidney functions normally. If however, rarely animals may show signs of kidney failure (hunched posture, piloerection, abnormal drinking and eating).

Uncommonly, agents may cause skin inflammation, thickening or flaking, hair loss or altered pigmentation, benign cysts, skin erosions or tumours. When the reagent is applied to a pregnant female, these possible adverse effects might apply to the offspring.

Where tumours develop, the majority (~85%) are expected to be small epidermal tumours which will have no significant impact on the animal's general well-being.

Repeated application of various agents in ethanol or acetone may occasionally cause excoriation of the skin.

Where tumours develop, the majority (~85%) are expected to be small epidermal tumours which will have no significant impact on the animal's general well-being.

How will you monitor for, control, and limit any of these adverse effects?
If adverse effects can't be prevented, how will you attempt to ameliorate their initial signs?

Limits are needed only where they impact directly on animal welfare. See general constraints for restrictions that will automatically form part of the licence. Examples of limitations include maximum dose or blood sample volumes (in ml/kg), maximum number of procedures, minimum intervals etc. You will need to detail how you will monitor animals post-surgery.

The limitations on severity and controls specified in this section need to be clear to personal licensees working under the project licence. They will use this information to judge whether or not they need to tell the PPL holder of a breach of severity under PIL SC 13 which may lead to a PPL SC18 notification. Animal care staff will use this information as a guide as to when to contact the personal licensee responsible for the animals, and as a guide to what action to take if no-one is available.

Monitoring must be specifically tailored for the procedure in question and take into account the stage or phase of the disease development and the rate of change of the animal's condition.

Establishment guideline documents should not be referred to as these do not form part of the licence. Relevant information that specifies procedures, controls, monitoring, endpoints in protocols should be included in all relevant protocols.

External published guidelines should normally be considered when determining procedural steps and end points (e.g., LASA Guidelines).

Example: If excoriation of the skin is seen, skin painting will stop until the skin is completely healed. Tumours will be measured at appropriate intervals and animals will be monitored by daily physical examination during the expected critical periods of tumour growth.

What are the humane endpoints for this step?

This would be the point at which you would kill the animal to prevent further suffering.

End points should be specified in the licence. Endpoints should not be 'determined by the NACWO or NVS', although indicating when their advice will be taken is helpful – see examples below.

Humane endpoints should relate to the adverse effects. They should be relevant and justified i.e., if the protocol is classified as mild, the humane endpoints should not be set at moderate severity level.

If harms are seen, then endpoints should be set to minimise suffering but also enable scientific outputs to be achieved.

Example: If the healing process is incomplete in X days or excessive scar tissue develops the animal will be killed.

Example: If the mean diameter of single tumours in rats exceeds 25mm the animal will be killed. Animals will be killed earlier if the tumour ulcerates or impedes any vital function (e.g., locomotion, vision, mastication, excretion). If a large number of small tumours or cysts accumulate in an area that impedes vital function, the animal will be killed.

Protocol 1: Fate of animals

What will happen to animals at the end of this protocol?

Select all that apply

- Killed

Will you be using non-schedule 1 killing methods on a conscious animal?

Yes/No

For each non-schedule 1 method, explain why this is necessary.

Answer YES to the question ONLY if the animal is conscious when it is killed e.g., decapitation of conscious neonates.

All methods of killing must be stated either in the protocol steps (e.g., a standalone step for decapitation) or as part of final killing step e.g., schedule 1 methods, exsanguination, completed by a schedule 1 method, perfusion fixation under terminal anaesthesia etc. The 'Fate of animals' section does not appear in the licence.

- Kept alive
- Continued use on another protocol in this project

Please state the relevant protocol.

Experimental protocols should typically not include continued use on to further experimental protocols.

Example: GA and wild type mice may continue to be used on protocols 2,3 and 5 of this project and on other projects with authority for continued use of animals of this type.

Example: Rat with a surgically implanted jugular catheter may continue to be used on other projects with authority to use animals of this type.

- Continued use on other projects

Protocol 1: Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.
Consider the cumulative effect of any combinations of procedures that you may carry out.

Applicants can also explain here any general husbandry measures they will take to minimise contingent harms, such as housing NHPs in social groups.

Example: Mice are immunised with an immunogenic substance and/or a pathogen [descriptors]. In some cases, mice will be given test substances daily for up to a week, typically by one or two enteral or parenteral routes; on rare occasions by a maximum of three routes. Control animals may not receive any substance administration. Blood samples will be taken, typically weekly and all animals will be killed within 3 months of immunisation. The test substances will already have been tested to ensure that the dosing regimen does not cause toxicity.

Example: Animals will receive a stereotaxic injection of a viral vector expressing optogenetic constructs into the brain, followed by recovery time of 1 to 6 weeks.

Animals should make an uneventful recovery. Then animals will be anaesthetised and decapitated for ex vivo brain slice preparation.

Describe the general humane endpoints that you will apply during the protocol.

These will be in addition to the endpoints stated for each step.

General humane endpoints are not required if the humane endpoints are clear for each step and there is no cumulative or other harm. General humane endpoints are in addition to those for each step and should limit cumulative harm. They should be relevant and justified (i.e., for mild protocol humane endpoints should not have suffering in moderate level) and should be consistent with the humane end points for each step.

Example of moderate severity general humane end points: Any animal that shows deviation from normal health (such as piloerection, hunched posture, abnormal gait, inactivity or inappetence) with be monitored more frequently and supportive treatment provided such as warming and wet mash. Should the signs persist for a period of 24 hours the animal will be humanely killed using a Schedule 1 method. In addition, any animal that loses 15% of its body weight compared to age matched controls will be killed.

Protocol 1: Experimental design

What outputs are expected to arise from this protocol?

For example, test results, phenotypic information, or products.

Examples of outputs include tumour development and growth (rate of growth, volume, and number of metastatic sites); plasma cytokine levels; histological findings such as tumour vascularisation; genetically altered animals.

Example: The purpose of the protocol is to determine the potency of the test vaccine, which is calculated from the proportion of mice protected against toxin challenge in the test vaccine groups relative to the proportion of mice protected in the reference vaccine groups.

Will this protocol generate quantitative data?

Yes/No

Will your experimental design be determined by a regulatory guideline?

No

Where relevant, explain how and when pilot studies will be used.

Further advice available at NC3Rs Guidance on Conducting a Pilot Study. Any pilot studies that do not use the mandatory step will need either a separate protocol or be included as alternative (optional) step within the mandatory step.

How will you choose different experimental groups?

For example, controls, dose levels, satellites etc.

Example: The effect of anticancer drugs in animals with induced or implanted tumours will be tested typically at two, occasionally up to four, dose levels around the expected effective dose. Usually this involves no more than 3 animals per group. Doses will be adjusted when no effects, or rarely seen adverse effects, are observed. Unless there are good reasons otherwise, the experiments will be designed to compare dose levels.

How will you choose control groups?

Provide a robust scientific justification for controls with significant suffering such as sham surgery controls or untreated infected controls.

Clearly describe how control groups, including sham surgical controls, are used and the steps they will undergo. Provide a robust scientific justification for the use of sham surgical controls.

Example: Controls are needed in all experimental approaches to model confounding variables in common with the treatment group. We have considered confounding variables outside direct experimental manipulation include animal age, sex, microbiota, cage in which the animal is house, room temperature and humidity. Control animals will be randomly allocated from the same pool of animals as treatment groups.

How will experiments and data analysis be randomised and blinded?

Example: We will make appropriate arrangements to randomly assign animals to experimental groups using block-randomisation tools (e.g., the NC3Rs EDA and blind studies in accordance with the PREPARE guidelines and will plan and conduct studies to enable them to be published according to the ARRIVE guidelines.

How will you minimise variables to ensure reproducibility?

Example: Limit animal variation by using strains of the same background: sex, age, weight.

Example: Limit nuisance variables by standardising the time of day procedures are performed, or data are collected.

Example: Use standard protocols in experimental procedures and data analysis

How will you determine group sizes?

You should reference POWER calculations you have made, if relevant.

Power analysis is often useful in determining group size but not always applicable. Consider whether factorial designs are suitable. Specialist advice on experimental design and statistical analysis of results is usually widely available at establishments.

Example: Where relevant, factorial experimental designs will be used, rather than the one-thing-at-a-time approach, to maximise the information obtained from the minimum resource. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 25%. Otherwise, we will use our previous experience (ours, or from the literature) to select sample sizes. In terms of the numbers of animals required, we expect that 6 to 8 animals per treatment group should be sufficient to obtain the required results. However, because of the difficulty in obtaining satisfactory data from the very small dorsal root ganglion cells of C-fibre neurones, we expect to have to use rather greater numbers of animals per group to obtain satisfactory results: at this stage we are unable to provide a reliable estimate.

How will you maximise the data output from the animals you use on this protocol?

Example 1: Where possible up to 3 test vaccine batches are tested together in the same assay to maximise the use of the reference vaccine and control groups. Thus, minimising the use of animals overall.

Example 2: At post-mortem we will take and store as many tissues as possible and make them available to other researchers.

Protocol 1: Protocol justification

Why is each type of animal, experimental model, and/or method selected for this protocol:

a) the most appropriate scientific approach?

Scientifically justify the choice of animals, the models, and methods in relation to the programme of work. Specific robust justification is required for severe procedures.

Example: We intend to use three models of peripheral neuropathic pain:

1) chronic constriction injury (CCI or Bennett model)

2) partial sciatic nerve ligation (PSL or Seltzer model)

3) L5/L6 (rat) or L4/L5 (mouse) spinal nerve ligation model (SNL or Kim & Chung model)

Each of these models has slightly different characteristic effects and we may need to use all of them to answer our particular scientific question. The SNL model has the advantages of a more consistent site and extent of ligation than the CCI or PSL models, and of having separate injured and intact spinal segments, but the disadvantage of requiring more extensive surgery.

b) the most refined for the purpose?

Refer to or consult published guidelines to ensure maximum refinement of models e.g., IMPROVE guidelines for stroke models or other expert working group papers.

For each model and/or method, what is the scientific need for the expected clinical signs?

You need to explain the degree of pathophysiological changes that are scientifically necessary to achieve your scientific objectives. How do these correlate with the clinical signs in the animals? Why, scientifically, do the animals need to suffer to this degree? Why can you not achieve your scientific objectives with an earlier endpoint, or without the animals showing clinical signs at all?

Example: These are models of peripheral neuropathic pain and animals will show behavioural signs of spontaneous pain include guarding, excessive licking, and lameness in the ipsilateral hind paw. We require clinical signs hyperalgesia and allodynia so that we can measure them and assess response to treatment.

Example: Animals will show clinical and behavioural signs. In our experience there is good correlation between hepatic immunophenotype and necroinflammation, and clinical signs such as temporary inactivity and affected gait. These clinical cues are more indicative of level of injury in the animals than serological parameters such as ALS/AST. We rely on these physical signs to demonstrate that individual animals are experiencing hepatic injury in response to their individualised treatment regimen.

Why scientifically do the animals need to suffer to this degree?

Example: The inflammatory nature of the model is such that to get meaningful results, some erythema and pruritus may be necessary. We aim for a model with the minimum of signs consistent with the particular experimental needs.

Why can't you achieve your scientific outputs with an earlier humane endpoint, or without animals showing any clinical signs?

An example of a scientific endpoint would be blood glucose of > 300 mg/dl. Such animals would show some polyuria and polydipsia but no significant weight loss. A humane endpoint might be, for example, a hunched appearance with reduced locomotion or a particular score on a clinical/ welfare assessment sheet.

Will you be administering substances for experimental purposes?

Yes/No

How will you assess the suitability of these substances, and minimise the unnecessary harms arising from their administration given the particular strain or type of animal you will be using?

When assessing suitability, state how you will consider toxicity, efficacy, and sterility.

Examples include pilot studies, dose range finding studies, reliance on literature, using previous experience. Checks on potential toxicity and sterility are particularly important for novel test substances. How do you ensure suitability for administration into animals? e.g., in vitro tests, appropriate pH etc.

How will you determine an appropriate dosing regimen?

Include routes, dosage volumes, frequencies, and durations.

Describe how you will determine appropriate dosing regimens e.g., pilot studies, literature review, previous experience, or collaborators.

Example: If toxicity is unknown the substance will initially be tested on unoperated animals. Dose setting, at a low dose in no more than two animals initially will be undertaken on a dose finding protocol. If no toxicity is seen, a further two animals may be tested at a higher dose and so on until an appropriate pharmacological dose level is reached. If the initial dose produces evident toxicity, doses will be reduced and retested.

Use of animals

Cats, dogs, and equidae

What are the scientific reasons for using cats, dogs, or equidae in your project?

A licence cannot be granted unless your scientific objectives or research questions can only be achieved or answered by the use of cats, dogs or equidae. This includes instances when it is not practicable to obtain other types of animals.

Explain why you need to use cats, dogs or equidae. A project licence cannot be granted unless the Secretary of State has verified that the purpose of the programme of work in the licence can be achieved:

- only by the use of cats, dogs or equidae; or
- only by the use of cats, dogs or equidae and other animals which it is not practicable to obtain.

The availability of background data or ‘the usual species of choice’ are not adequate justifications in themselves.

Note that applications proposing the use of cats, dogs, or equidae in procedures classified as severe or animals containing human material classed as Category 2 or 3 by the Academy of Medical Sciences will be referred to the Animals in Science Committee (ASC) for additional advice.

Non-human primates

Why do you need to use non-human primates, rather than any other type of animal, to achieve your objectives?

Explain why the purposes of the programme of work cannot be achieved by using species that are not primates.

Explain why the project is:

- translational or applied research for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man; or
- the development, manufacture or testing of the quality, effectiveness, and safety of drugs for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man; or
- basic research; or
- research aimed at preserving the species of animal subjected to regulated procedures.

Note that applications proposing the use of non-human primates in procedures classified as severe will be referred to the Animals in Science Committee (ASC) for additional advice

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Are any of these non-human primates endangered?

Endangered animals are any of the species listed on Annex A of Council Regulation 338/97 and are not bred in captivity.

Yes/No

Why can't you achieve your objectives by using non-human primates that are not endangered?

Also explain how you will comply with other regulations including CITES.

Provide relevant details.

Explain how the project is for one of the permitted purposes.

*The permitted purposes for the use of endangered non-human primates are: * translational or applied research for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man * the development, manufacture or testing of the quality, effectiveness and safety of drugs for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man * research aimed at preserving the species of animal subjected to regulated procedures.*

Applications that propose the use of wild-caught primates must be referred to the ASC for additional advice.

Might any of these non-human primates be wild-caught?

Yes/No

Why can't you achieve your objectives without using wild-caught non-human primates?

Applications that propose the use of wild-caught primates must be referred to the ASC for additional advice.

Purpose bred animals

Will all animals used in your project be purpose bred?
This means animals that have been bred primarily to be used in regulated procedures or for the use of their tissues or organs for scientific purposes.

Yes/No

Where will you obtain non-purpose bred animals from?
Consider the source of all animals you plan to use, as this information will help to assess the impact on the scientific output and the quality of the animal.

Provide scientific justification for using Schedule 2 species that are not purposed bred. Any non-Schedule 2 species should be from a source that can provide animals of a sufficient quality to produce satisfactory results.

Why can’t you achieve your objectives by only using purpose bred animals?

A scientific reason is required for using non-purpose bred Schedule 2 species.

Endangered animals

Will you be using any endangered animals, apart from non-human primates?
Endangered animals are any of the species listed on Annex A of Council Regulation 338/97 and are not bred in captivity.

Yes/No

Why can’t you achieve your objectives without using endangered animals?
Also explain how you will comply with other regulations including CITES.

Applications to use endangered animals will be referred to the Animals in Science Committee for additional advice.

Explain how the project is for one of the permitted purposes.

*The permitted purposes for the use of endangered animals are: * translational or applied research for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man * the development, manufacture or testing of the quality, effectiveness and safety of drugs for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man * research aimed at preserving the species of animal subjected to regulated procedures.*

Provide relevant details.

Animals taken from the wild

Will you be using any animals taken from the wild?

Yes/No

Why can't you achieve your objectives without using animals taken from the wild?

In this context an animal 'taken from the wild' means a previously free-living animal that has been captured or otherwise brought under the control of man:

- whether or not it is to be kept in captivity for any appreciable length of time;
- whether or not it is physically taken away from the place of capture / 'the wild';
- whether a physical trap or device is used to take the animal, or any other means is used to bring it under the control of man (for example, picked up in the hand).

Special conditions apply to the use of animals taken from the wild and a variety of requirements (e.g., relating to capture, animals that are found to be injured or in poor health on capture) need to be covered in the answers below to ensure these are met. Stray animals cannot be used and there are restrictions on the use of other types of animals, such as feral animals.

The Advice Notes 'Working with animals taken from the wild', 'Re-homing and setting free of animals' and 'Use, keeping alive and re-use' also provide helpful information for animals taken from the wild.

Example: Wild rats are required as we are investigating the incidence of a rodenticide resistance gene in wild populations.

How will these animals be captured?

Explain how each method is the most refined for the animal type or purpose of the study. Also include any relevant considerations around trapping, including the frequency of checks and trap positioning.

Provide relevant details.

How will you minimise potential harms when catching these animals?

Provide relevant details.

Will your capture methods catch non-target animals?

Yes/No

How will you minimise the risk of capturing non-target animals, including strays and animals of a different sex?

Example: We capture birds only in nest boxes. The nest box entries are designed to only fit the species we want to catch. The nest boxes are checked on a schedule as to minimise disturbance for the bird. Only on a few occasions is another species present (e.g., nut hatch, bat), which we will not catch, but leave undisturbed until after the breeding season.

What will you do with any non-target animals that you capture?

Example: On the rare occasion other avian species are caught in the mist nets, they will be carefully checked and released immediately.

How will you ensure the competence of any person responsible for the capture of animals?

Example: Wild birds will be captured by people with a current BTO ringers' permit allowing unsupervised capture of the relevant species

How will you examine any animals that are found to be ill or injured at the time of capture?
Include details about what will be done with these animals after they have been examined.

Example: Assessment of injury and body condition will be carried out at the earliest possible opportunity after capture. This is done before the application of any regulated procedures. Birds will be weighed. Any bird with a weight that deviates negatively from the normal distribution will not be sampled (less than 20g for adult house sparrows)

Will a veterinary surgeon perform the examination?

Yes/No

How will you ensure the competence of the person responsible for making this assessment?

Describe the process for ensuring the person making the assessment has the necessary knowledge, skills, and competency to determine the appropriate action on capturing an ill or injured animal.

Is it necessary to use animals that are injured or in poor health during your project?

Yes

Explain why it is scientifically necessary to use animals that are injured or in poor health during your project.

Provide relevant details.

If sick or injured animals are to be treated, how will you transport them for treatment?
Include how you will ensure that any potential harms during their transport will be minimised.

Provide relevant details.

If sick or injured animals are to be humanely killed, which methods will you use?

Provide relevant details.

Will animals be marked, or otherwise identified, during the project?
Consider both regulated and non-regulated procedures in your answer.

Yes/No

How will animals be identified?
State which methods may cause more than momentary pain, distress, or lasting harm to an animal.

Provide relevant details.

Will any devices be attached to or implanted in animals during this project?
For example, any device used to identify, track, and monitor an animal's behaviour in its natural habitat.

Yes/No

How will any adverse effects from a device's attachment or implantation be minimised?

Examples include ensuring that any device is as small as possible or the use of break-away collars.

How will you locate and recapture the animals or otherwise ensure the devices are removed at the end of the regulated procedures?
If devices will not be removed, explain why it is not required.

Provide relevant details.

If animals will not have devices removed, what are the potential effects on them, other animals, the environment, and human health?

Provide relevant details.

I confirm that I have, or will have, all necessary permissions from other regulators in place before commencing any work involving animals taken from the wild.

Yes

Feral animals

Will you be using any feral animals in your project?
A feral animal is an animal living in the wild but descended from domesticated individuals.

No

Other considerations

Neuromuscular blocking agents (NMBAs)

Will this project involve the use of neuromuscular blocking agents (NMBAs)?

Yes/No

Why do you need to use NMBAs in your protocols?

See Appendix H of the Guidance.

In addition, ensure that the use of neuromuscular blocking agents (NMBAs) is specified in the relevant protocol(s). You will need to show administration of neuromuscular blockers as a separate step and use the code 'AD' for all procedures conducted under neuromuscular blockade.

Ensure that the scientific need to use them is explained clearly in the action plan.

What anaesthetic and analgesic regime will you use?

Provide relevant details.

How will you ensure that animals have adequate ventilation?

Provide relevant details.

How will you minimise pain, suffering, and distress for an animal under the influence of an NMBA?

Provide relevant details.

How will you monitor the depth of anaesthesia?

Provide relevant details.

How will you ensure there are sufficient staff present throughout the use of NMBAs (including during recovery periods) who are competent to use them in these types of animals?

You need to provide evidence of existing competence of staff or how such competence will be obtained.

Explain the agreed emergency routine at your establishment that covers potential hazardous events (such as a power failure).

Provide relevant details.

Re-using animals

Why do you intend to re-use animals?
Explain how you will balance the needs of refining and reducing animal use before making your decision.

Provide justification for re-use based on a positive balance of reduction and refinement.

What are the limitations on re-using animals for this project?
For example, there may be a maximum number of times that an animal can be re-used, or a set of performance standards that requires a limit on re-use.

Consider the welfare and scientific considerations that will be used to determine suitability for re-use and the criteria that will be used by the veterinary surgeon to determine that animals can be re-used, including any limitations on the period of time that the animal will be held under the supervision of the NVS/VS.

Describe controls on re-use, for example:

- maximum number of times; and/or
- performance standards e.g., patency of a cannula;
- humane end-points in the form of behavioural and/or physiological indicators that animals are suffering as a result of long-term laboratory housing or as a result of being re-used.

Commercial slaughter

Will you send any farm animals to a commercial slaughterhouse at the end of their use?

Yes/No

How will you ensure that these animals are healthy and meet commercial requirements for meat hygiene to enable them to enter the food chain?
Include any relevant information about drug withdrawal times.

Consider the requirements under ASPA section 17A. Complete the following:

<<<INSERT animal type(s) HERE>>> may be sent directly to slaughter at a registered slaughterhouse at the end of their use provided that:

- The animal is healthy and meets the commercial requirements for meat hygiene to enable them to enter the food chain. They must not be infected with any notifiable disease and comply with the relevant substance withdrawal times;
- While kept alive at <<<INSERT place HERE>>> pending transport to the slaughterhouse, the animal is kept in an appropriate social group under the supervision of a veterinary surgeon;
- The animal is appropriately identified and is transported in accordance with the relevant legalisation.

Animals containing human material

Do you intend to use animals containing human material in experiments classed as Category 2 or 3 by the Academy of Medical Sciences?

Yes/No

Keeping animals alive

What types of animals will you keep alive?

See the Advice Note on Use, keeping alive and re-use.

Examples:

- Mice that have suffered actual severity that is no more than mild and are not suffering at the end of their use
- Dogs with implanted telemeters that are not expected to cause lasting harm
- Rats with implanted cannulae – for immediate transfer to the maintenance step in protocol x as re-use.

What criteria will the veterinary surgeon, or competent person trained by a veterinary surgeon, use to determine whether animals can be kept alive?

Provide relevant details.

Are there any limitations on the period of time that animals that have been kept alive can be held under the supervision of the veterinary surgeon?

Provide relevant details.

Setting animals free

How will an animal's health be assessed to determine whether it can be set free?

Complete the following:

<<<INSERT animal type(s) HERE>>> may be set free at the end of the series of regulated procedures conducted under the authority of protocol <<<INSERT protocol number(s) HERE>>> provided that the following actions have been taken:

- <<<INSERT actions to ensure the state of health allows the animal to be set free HERE>>>

Will a veterinary surgeon perform this assessment?

Yes/No

How will you ensure the competence of the person responsible for assessing whether animals can be set free?

Provide relevant details.

How will you ensure that setting animals free will not be harmful to other species, the environment, and human health?

- <<<INSERT actions to ensure that the setting free of the animal poses no danger to public health, animal health or the environment HERE>>>

Will you rehabilitate animals before setting them free? If so, how?

Provide relevant details.

Will you attempt to socialise any animals that you have set free? If so, how?

- <<<INSERT actions to ensure socialisation of the animal on being set free HERE>>>

How will you prevent inadvertent re-use of animals that have been released at the end of procedures?

Provide relevant details.

If animals are lost to the study or not re-captured, how will you determine whether your project is complete?
This information is important to ensure that the use of these animals is recorded in the return of procedures and is considered when determining the actual severity of your protocols.

Provide relevant details.

Rehoming animals

What types of animals do you intend to rehome?
Also state the protocols on which they would have been used.

<<<INSERT animal type(s) HERE>>> may be rehomed at the end of a series of regulated procedures provided that the following actions have been taken:

How will you make sure that an animal's health allows it to be rehomed?

<<<INSERT actions to ensure the state of health allows the animal to be re-homed HERE>>>;

How will you ensure that rehoming does not pose a danger to public health, animal health, or the environment?

<<<INSERT actions to ensure that the rehoming of the animal poses no danger to public health, animal health, or the environment HERE>>>;

_____

What scheme is in place to ensure socialisation when an animal is rehomed?

<<<INSERT actions to ensure socialisation of the animal on being re-homed HERE>>>;

_____

What other measures will you take to safeguard an animal's wellbeing when it is rehomed?

<<<INSERT any other measures to safeguard the animal's welfare on being re-homed HERE>>>.

_____