**Important:**

- Do not copy and paste from other documents if it affects formatting
- Turn off auto numbering. See the ASPeL Quick Start Guide for project licence applicants
- Put all pictures, diagrams & appendices etc as separate attachments, do not embed in text

Home Office

**ASPeL PROJECT LICENCE APPLICATION TEMPLATE – GENERAL LICENCE**

# UNDER THE ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

*You will be presented with a drop down box to complete to specify if your application is 'New' or an 'Amendment' request. (Guidance is provided in the Quick start Guide for Applicants)*

**PROJECT TITLE**

A1.1 This should describe the theme of the work and define the area of interest in a way that is likely to remain valid for the duration of the licence. There is no character limit but it is sensible to keep this reasonably short.

A1.2 *Examples:*
*Mechanisms of heart regeneration and development*
*New treatments for peripheral arterial disease*

## A. PROJECT LICENCE HOLDER

Under the Animals (Scientific Procedures) Act 1986, section 5, a project licence is granted by the Secretary of State which specifies a programme of work and authorises the application, as part of that programme, of specified regulated procedures to animals of specified descriptions at a specified place or specified places. The project licence holder is responsible for the overall implementation of the programme of work and for ensuring that the programme is carried out in compliance with the conditions of the licence.

| | |
|---|---|
| Title (e.g. Professor, Dr, Mr) | |
| Surname | |
| Forename(s) | |
| Qualifications | |
| Position or appointment | |

If you have previously been known by another name or names, give the name(s):

| | |
|---|---|
| Surname | |
| Forename(s) | |

**Contact details**

| | |
|---|---|
| Address for correspondence<br>*This will normally be the address of the establishment where you are working and must be within the UK* | |
| Post Code | |
| Telephone number and extension | |
| Mobile phone number (optional) | |
| E-mail address (please use criminal justice secure mail (CJSM) if you have one) | |
| *It is now possible to encrypt personal and other possibly sensitive information sent to, or received from, the Home Office by e-mail through the CJSM system. CJSM facilitates routine secure e-mail correspondence with minimal inconvenience to users. Detailed instructions for registering can be read on the CJSM website at https://www.cjsm.net/* | |

**Your relevant knowledge, skills and experience**

Provide brief details of your knowledge, skills, experience and current role. Include all relevant roles in research involving animals. Explain why you are a suitable person to take responsibility for this programme of work.

A2.1 This section is particularly important if you have never held a project licence before. Please look at the Guidance para 5.2 (page 39) 'Who can hold a project licence' and make sure you can comply with all the requirements.
https://www.gov.uk/government/publications/operation-of-aspa

A2.2 For established project licence holders with a good track record of achievements in the field it will be sufficient to summarise how you have achieved benefits from previous projects or your current work.

A2.3 For other applicants, you must be in a position to undertake responsibility for the whole programme of work and have the appropriate training and education. Explain **briefly**:

- why you are the most suitable person in the research group, department or company to manage the project;
- how others will help you manage the project (if applicable);
- your relevant scientific knowledge;
- that you have specific knowledge relating to all the species of animal that will be used in the programme of work (see advice on training requirements in the next section); and
- how you have competence in experimental design and data analysis or access to these skills.

A2.4 Explain how you are able to take overall responsibility for the proper design and conduct of the whole programme of work including:

- the scientific direction, management and control of the programme of work;
- compliance with the conditions of the project licence;
- the supervision, training, conduct and performance of personal licence holders working under your control both within the project and their personal licence authorities;
- ensuring that the programme of work is done according to the principles of the 3Rs;
- experience of managing programmes of work especially under a UK project licence.

A2.5 If you have held a project licence before and are now proposing to use a new species or include work falling under a different personal licence category than your previous work (e.g. your application now includes surgical procedures) then you must add details of how you have gained the relevant additional knowledge and experience required.(see Appendix 6 Draft Advice Note on training requirements Section 2.3)

A2.6 Whether you are a new applicant or have held a project licence before, consider:

- how long you have worked with research animals and with which species;
- how long you have held a UK project licence and/or personal licence;
- how long you have carried out experiments on animals if not in the UK;
- publication record in the field.

A2.7 *Example for a new project licence holder:*
*I gained a BSc degree in farm animal science from the University of XXX in 2005 and a PhD in bovine nutrition in 2009. Subsequently I held the post of a post-doctoral research associate on a cross disciplinary project relating milk yield to nutrition in commercial farms. Resultant findings led to a BBSRC-funded grant, on which I was employed as a researcher co-investigator and was responsible for directing the work of a post-graduate researcher and planning the experiments for the group. I have a total of 9 years' research experience in the field of farm animal nutrition, have co-authored a number of published papers and have developed expertise in a wide range of experimental and data handling techniques. While I have no direct experience of research involving laboratory animals, I will be mentored by Professor Black who is an experienced project licence holder and will be guided by the NACWO and NVS, particularly in matters relating to the 3Rs.*
*I have attended all relevant training courses for project and personal licence holders and will be supported by an experienced team of animal technologists with experience in carrying out the proposed regulated procedures.*
*I have recently started my independent academic career, obtaining a 5 year fellowship position funded by XXX. As the Principal Investigator on this grant, it is most appropriate for me to take responsibility for the research involving animals.*

*Example for an established project licence holder*
*I am an established project licence holder having held three previous project licences to cover this ongoing programme of work. I have experience of all procedures and species to be used in this application. In my current licence, I have published five papers in peer-reviewed scientific journals and expect to publish another five in the next twelve months. We have made a number of 3Rs gains including replacing an in vivo model with an in vitro one to cover around 20% of the programme.*

**Module Training**

*You will be required to enter details of module training appropriate to your programme of work, you will need the module number, date passed, accrediting body and certificate number and species where applicable.*

*Complete a separate template for each module appropriate to your programme of work. If you are seeking an exemption, complete a separate template for each module from which you are seeking an exemption (see ASPeL User Guidance and Guidance on the Act).*

A3.1 Full details of the mandatory training requirements for project licence applicants are in chapter 9 of the Guidance, summarised in Figure 4:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291350/Guidance_on_the_Operation_of_ASPA.pdf

A3.2 For exemptions and/or unusual scenarios discuss with your NTCO and see the Appendix 6 Draft Advice Note on 'Training Requirements'.
For example, if you have held a project or personal licence within the last 5 years you may be exempt from some or all of the mandatory training requirements.

A3.3 Although a personal licence is not a requirement for holding a project licence you will need to provide evidence of appropriate mandatory (or equivalent) training.

A3.4 Common problems with this section are:
a) Not completing a module PILC template when surgery is included in the programme of work.

b) Including certificates dated more than 5 years ago – current or recent licence holders may be able to claim exemption instead;
c) Experience with all species requested is not included – you need to have appropriate knowledge of and training for all the species on your project application whether or not you propose to carry out regulated procedures yourself.

A3.5 Regarding training for using unusual species, see also Advice Note 'Working with animals taken from the wild':
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf

| | |
|---|---|
| Module number | *For example: PILA(skills & theory), PILB, PILC* |
| Description of animals | *mice, rats* |
| Exemption sought Yes/No | *Yes* |
| Provide evidence to justify the exemption request if applicable | *I hold/have held* a project licence which expires/expired* on xx/xx/xxxx. The project involved surgical/non-surgical* procedures on [species]*<br><br>**delete as applicable* |
| Date passed | |
| Accrediting body | |
| Certificate number | |
| Other relevant experience and training | |

**Expertise and other resources, peer review and funding**

- Describe the expertise, staffing, facilities (including housing, husbandry and care conditions for the animals and equipment) that are available to you
- State whether the proposed work has been peer-reviewed and if so, by whom
- State how the project will be funded and indicate to what extent funding has already been confirmed

A4.1 This section is to provide information for the inspector to use when assessing the likelihood that the stated benefits will be achieved.

A4.2 Make sure you address each of the points in the purple box above. It is not essential that you have funding for the whole five years in place, but please indicate how long you expect your current funding to last. If you have no or little funding, explain your plans for funding the programme of work and comment on your track record of securing funding for this kind of work, where applicable. We need to be clear that funding is or is likely to be available This is to provide reassurances that you won't run out of funding before benefits have been achieved, thereby wasting animals and causing unjustified suffering. This is particularly important for longer term studies, those resulting in severe severity or using special species;

A4.3 Consider:resources provided by your current research team, including the facilities

available and any specialised equipment (e.g. 2 photon microscope or MRI) for this particular project;

A4.4 *Example 1: Technical expertise acquired through 31 years relevant experience. The research team consists of me, as principal investigator, an average of two post-doctoral scientists, one PhD student and one laboratory technician. Laboratory and animal facility space is made available at the University of XXX. I possess the required tumour biology equipment and have access to shared facilities for the production and maintenance of genetically altered animals. I am personally experienced in all the procedures to be applied to the animals. Funding has been obtained from Research Council A and Charity B after peer review. It is sufficient for the duration of this project.*

*A4.5 Example 2: The bovine nutrition group at XXX University consists of 3 PIs with a wide range of skills and expertise, and extensive experience in planning and performing in vivo studies. All experiments will be performed in the facility at XXX, which provides excellent housing, husbandry and care conditions for the animals including specialised large animal surgical suite. Surgery will be performed with the advice and guidance of Dr Pink, a large animal surgeon at XXX who has experience of this type of surgery and excellent success rates. The NVS has extensive experience in bovine anaesthesia. The dosing protocols will be optimised with the assistance of Prof Green and his group members, who have experience of using this method successfully. The work outlined in protocols 1-3 is funded by a Career Development Fellowship from XXX, and underwent rigorous peer review prior to funding. The work outlined in protocol 4 has been submitted as a XXX project grant. If funding for the work in protocol 4 is not secured, this work will not go ahead.*

**Personal licences**

| Provide the number of your current or previously held ASPA personal licence. |
|---|
| A5.1 This information is used to support requests for exemption from further mandatory training. Note: It is not essential for a project licence applicant to hold a personal licence.<br><br>A5.2 Only refer to UK personal licences held within the last 5 years. It is helpful to list the species you have authority to use on your personal licence and the authorised categories (A, B, C etc).<br><br>A5.3 *Example: I hold personal licence number I123B67S which authorises Category ABC for rats and mice.* |

**Project licences**

| Provide the number(s) and expiry date(s) of your current or previously held ASPA project licence(s). |
|---|
| A6.1 This should be project licences **you, personally** have held within the last 5 years.<br><br>A6.2 Do not list licences here where you have been a 'deputy' for the PPLh'. Experience, for example helping to manage *in vivo* work, can however be included to support 'your relevant knowledge, experience and skills' in the section above.<br><br>A6.3 We ask for this information as it will help to assess your track record as a project licence holder or to support a request for exemption from further training. |

**Duration of project**

Under the Animals (Scientific Procedures) Act 1986 section 5E(1), the maximum allowable duration of a project licence is five years.
*You will be required to specify the duration using a drop down box*

| Specify the duration of licence you require if less than five years. |
|---|
| A7.1 Licences that are issued for less than 5 years can be extended to the full 5 years on receipt of a suitable amendment request.<br><br>A7.2 Licences cannot be granted for more than 5 years or the duration extended beyond 5 years. |

**Alternative Contact details**

A8.1 This should be someone who can answer any scientific queries and someone the Home Office inspector can contact in your absence to discuss the programme of work (e.g. another faculty member who is likely, ideally, to remain in post for the duration of the licence).

| In your absence, who may we contact if we have any questions about the management of your project? | |
|---|---|
| Surname | |
| Forename(s) | |
| Position held | |
| Telephone number and extension | |
| E-mail address (*please use criminal justice secure mail (CJSM) if you have one*) | |

## B. PLACE(S)

Under the Animals (Scientific Procedures) Act 1986 section 5(2), this must be a place at which a person is authorised by a Section 2C Licence (Establishment Licence) to carry on an undertaking involving the applying of regulated procedures to protected animals (a 'user' establishment).

### Primary availability

| | |
|---|---|
| 2C (PEL) Licence number: | X1234ABCD |
| Name of licensed establishment: | |

### Additional availability (if any)

If you intend carrying out the regulated work specified in this licence at more than one licensed establishment, you will be required to complete an additional template for each establishment. You should note that the relevant parts of this application must be approved by the Animal Welfare and Ethical Review Body at each additional establishment. *(Guidance is provided in the Quick start Guide for Applicants* https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/579962/PPL-Quick-Start-Guide-Applicants.pdf*)*

| | |
|---|---|
| 2C (PEL) Licence number: | |
| Name of licensed establishment: | |

| Explain why you need this additional availability<br>Indicate whether you intend to move animals between establishments during the course of a series of regulated procedures and, if so, describe the reasons for such transfers. |
|---|
| B2.1 **Note** – this part of the application form has a restricted character limit. To provide all the information needed, you may need to include some of it in the Plan of Work.<br><br>B2.2 Information in this section is used by the inspector in the harm-benefit analysis as it a) contributes to the assessment of whether a successful outcome is likely; and b) the movement of animals between sites may contribute to the overall harms.<br><br>B2.3 Explain which protocols or parts of protocols will be undertaken at sites of additional availability and why work needs to be done there, for example specialised equipment is required.<br><br>B2.4 If you need to move animals between primary and additional establishments, you should explain:<br>• **why** you need to move live animals;<br>• **when** in the series of regulated procedures they would be moved;<br>• **how** you will ensure that they will be in a suitable condition to travel;<br>• **what** arrangements will be made to assure their welfare during transport and to comply with relevant applicable transport legislation (e.g. Welfare of Animals in Transport legislation), particularly if they are being moved after the start of regulated procedures;<br>• **how** the proposed movement may impact scientific delivery and what control measures are in place to minimise any potential adverse impact arising as a result of this movement e.g. allowing an acclimatisation period of X weeks before regulated procedures are carried out.<br><br>B2.5 Genetically altered rodents, zebra fish and Xenopus spp are commonly moved after production at one facility for use at another. You do not need to include here details of such movements unless regulated procedures, apart from identification/genotyping, have been performed. Authority for such movements is provided in the Transfer of Animals Section later in the application form. |

| Person responsible for supervising the work at this additional establishment | |
|---|---|
| Title (e.g. Professor, Dr, Mr, Ms) | |
| Surname | |
| Forename(s) | |
| Address for correspondence<br>*This will normally be the address of the establishment where the supervisor is working and must be within the UK* | |
| Post Code | |
| Telephone number and extension | |
| Mobile phone number (optional) | |
| E-mail address (*please use criminal justice secure mail (CJSM)if you have one*) | |

**Places Other than a Licensed Establishment (POLEs) (if any)**

| List any place(s) that is not a licensed establishment where you intend to carry out regulated procedures. |
|---|
| B3.1 This section requires details of all regulated procedures done at places which are not included in an establishment licence, and not just those done to animals "in the wild". Note that if you are working with wild animals you also need to provide information as prompted in the section towards the end of Part D: 'Animals taken from the wild'.<br><br>B3.2 POLEs may include places such as inland waterways or farms. List POLEs either as specific locations, if known, or in more general terms. See Guidance para. 5.7.6 and Advice Note 'Working with animals taken from the wild'. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf<br><br>B3.3 Explain how you will ensure that the inspector can inspect procedures at a POLE – e.g. obtain consent from land owners.<br><br>B3.4 Note that a condition will be placed on your licence requiring you to agree the arrangements for notification of work at a POLE with your assigned inspector. You must agree these arrangements **before** you start work at a POLE. |

| Explain why do you need to undertake regulated work at this POLE |
|---|
| B4.1 You must provide a scientific reason why you need to work at a POLE. See also Advice Note 'Working with animals taken from the wild'.<br><br>B4.2 Explain why the procedures can't/shouldn't be done at a licensed establishment.<br><br>B4.3 Explain what procedures will be done at the POLE (i.e. which protocols/procedures) and why they need to be done there.<br><br>B4.5 If you need to start regulated procedures on animals at a POLE and then move these animals to an establishment, you should explain:<br>• **why** you need to move them;<br>• **how** you will ensure that they will be in a suitable condition to travel;<br>• **what** arrangements will be made to assure their welfare during transport, particularly if they are being moved after the start of regulated procedures;<br>• **what** impact such movement will have on the scientific data to be collected?<br><br>B4.6 Details about keeping animals alive and setting them free to the wild (if appropriate) should be included in the protocol.<br><br>B 4.7 Information about animals that are taken from the wild (or obtained from other non-establishment sources) and moved to an establishment **before any** regulated procedures are carried out should not be included in this section; instead this should be included in the Plan of Work and Source of animals sections. For details of the information required see section 3 of the Advice Note 'Working with animals taken from the wild'. |

## C. SCIENTIFIC BACKGROUND

The total response to this Part must not exceed 2000 words

### Background

To enable us to judge the relevance and value of the aims and objectives of your project we need to understand the current state of knowledge or product availability on which the proposed project intends to build

- For research projects: Summarise the current position in your area of work and explain how this project will help to advance knowledge or meet a clinical need.
- For testing or screening projects: Provide a summary of the relevant statutory requirements or regulatory guidelines.
- For service or production projects: Describe the likely demands for the service or product in the lifetime of the licence
- Where applicable, summarise your achievements under any previous related ASPA project licence. Explain the extent to which you achieved the stated objectives of your previous project and list publications or other significant outputs.

C1.1 The information in this section gives the inspector the background information they need to understand the context of the application within the relevant scientific field(s).

C1.2 Most applicants complete this section and other parts of Part C **after** deciding on their aims and objectives in Part D. This section must clearly explain the relevance and value of all of the aims and objectives stated in part D.

C1.3 **Suggested approach**

- **Briefly** set out the current state of knowledge on which the current project intends to build (how have you come to the start of the (new) five-year project?).
- Ensure the background is specific and relevant to the aims and objectives of **this** project – the background section should not be a detailed overview of the field and relevant literature.
- Include any **relevant** research not involving animals that contributes to the starting point for this project.
- Present key arguments concisely.
- Use references from your group and the work of others (and/or regulatory guidelines if appropriate) and outcomes of past work to support the main points stated and the models to be used.
- Ensure specialist acronyms relevant to your science are defined the first time they are used.
- For a disease-specific biomedical discovery programme, briefly explain the prevalence and severity of the condition, availability of treatments and why a different therapeutic approach is necessary.
- Refer to progress made under any previous project (including outputs, benefits and 3Rs advancements) that are relevant to this application. For example:
    - publications. A list of your recent publications can be provided as a supporting document and will not form part of the licence;
    - GA strains to be used in further research;
    - compounds advanced to development;
    - therapeutic or scientific approaches discarded;
    - products taken to clinical trials or approved;

- patents;
- contribution to policy initiatives.

**Benefits**

We have to carry out a harm-benefit analysis of the programme of work to assess whether the harm that would be caused is justified by the expected outcome, taking into account ethical considerations and the expected benefit to human beings, animals or the environment (ASPA section 5B(3)(d)).

To enable us to do that you must:

- Set out the expected benefits of your programme of work; and
- Explain why those benefits will be worthwhile

We need to understand:

- What data or product outputs will be generated by your programme of work
- Who will use those outputs (e.g. your group, other researchers, the pharmaceutical industry, clinicians, patients)
- How will the outputs be used (NB benefit might be to screen out)
- The short-term, medium-term and long-term benefits

C2.1 Most applicants complete this section **after** deciding on their aims and objectives in Part D. The contents of this section must clearly be linked to the stated aims and objectives.

C2.2 **Suggested approach:**

C2.3 Consider each of your objectives in turn and then draft a summary:

- ***What*** are the intended outputs of the project (data / products)?
- ***Who*** will use those data or products (your group, others at your establishment, others) and, if possible to estimate, how many will benefit?
- ***How*** will the outputs be used and disseminated? (Remember the benefit might be to screen out – e.g. identify substances at an early stage that have unacceptable side effects or prevent others following an unproductive line of enquiry and therefore using more animals unnecessarily in future).
- ***When*** might the benefits be expected to be realised if your project is successful – within the timeframe of the licence (short to medium term) or after it has finished (longer term)?

C2.4 Provide the benefits of your project only (not of other work going on in the field).

C2.5 The benefits should reflect the stated permissible purpose(s) at the start of Part D.

C2.6 Benefits might be:

- publications and presentations;
- patents filed;
- Y candidate compounds expected to be progressed to phase 1 trials;
- Z approaches to therapy expected to be discontinued;
- animal welfare gains;

- influence on public policy.

C2.7 **Points to consider**

C2.8 This section is critical in the harm-benefit analysis done by the inspector that has to be favourable before a licence can be granted.

C2.9 For details of the harm-benefit analysis that inspectors undertake, read Appendix I of The Guidance and the advice note 'Harm-benefit Analysis Process' on our website https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/487914/Harm_Benefit_Analysis__2_.pdf

C2.10 The benefits need to be:

- specific to this project. You can briefly outline potential future additional benefits this work may lead to, but it should be clear what you expect *this* project to achieve, as opposed to claims based on future work that is not part of this application;
- realistic;
- achievable in the (maximum 5 year) duration of the licence.

C2.11 Discovery research that advances knowledge in a particular field is perfectly acceptable as a benefit in itself. Outlining the potential for translation is useful in many cases, but it is not essential and such explanations should be brief.

C2.12 The expected benefits of a project should take account of:

- the starting point on which the project is built (explained in the background);
- the likelihood of success;
- the anticipated subsequent use of the outputs.

C2.13 If your project licence application involves work that was recently the subject of a grant application, you may find that there is useful information in the 'impact statement' for completing this section.

C2.14 **Likelihood of success**

C2.15 Ensure you give sufficient information, for the project as a whole and for each objective, for the Inspector to determine the likelihood of you achieving your purpose within the lifetime of the project. Factors taken into account in evaluating whether the benefits are likely to be achieved include:

- past performance – your or your group's previous project achievements as appropriate;
- novelty of the models & methods to be used. Applicants should indicate in the '3Rs' section how novel approaches will be evaluated so as to minimise unproductive animal use;
- chances of success with these models & methods.

C2.16 ***Example 1**: Epidermal stem cell self-renewal & differentiation*

*This work is expected to provide novel information about the properties of stem cell self-renewal and the pathways that regulate their differentiation. It will advance our knowledge of how molecular processes in normal tissue are mis-regulated in cancer. Pathways and factors involved*

*in tumourigenesis may be identified that could lead to the discovery of new strategies for treating non-melanoma skin cancer, one of the most common cancers in the world (Refs).*

*This work will increase our knowledge of how pathways regulating stem cell fate in healthy tissue may be misregulated in cancer, and whether they can be modulated either positively or negatively.*

*The work should provide valuable information on how the genes under investigation act on human tumour cells. Human cancer cells are used in grafting experiments and information obtained from them should have immediate application in patients.*

*This work will also advance fundamental scientific knowledge of stem cells and the genetic pathways that control regeneration of healthy and diseased skin.*

*Findings will be made available to other scientists through publication in peer-reviewed journals and presentations at scientific conferences and meetings. Under the previous project we published x papers (and 2 more are currently being written) and we presented our findings at y international scientific meetings.*

*The transgenic animals developed will be valuable and made available to other scientists interested in the development of anti-cancer therapies.*

*Likelihood of achieving these benefits:*
*All methods required to label stem cells or to regulate expression of the transgene in the epidermis are well established and I have been successfully using these methods on my current project licence. The specific regulators of the Myc-oncogenic system used are highly likely to be involved in cell cycle regulation so changes in their expression are very likely to affect tumour development. I have successfully proven this strategy by identifying a novel direct down-stream target of Myc, called XXX that is involved in Myc-mediated proliferation. Under my current project licence I have further shown that inhibition of XXX expression reduces tumour growth. This research has led to a patent filing in 2012. Thus, it is highly likely that we will identify novel potential anti-cancer drug targets with this approach within 5-10 years.*

C2.17 ***Example 2***
*The primary expected benefit is the generation of new knowledge, defining how endogenous progenitor cells behave in [specific organ] injury and repair. These data will be presented at national and international conferences, and published in academic journals and will provide a new appreciation of how cell behaviour can be controlled to limit [organ] pathology and aid regeneration, at both the cell and tissue levels.*

*The long-term potential benefits of this study are that data generated may have far-reaching implications for the treatment of [disease condition], both in humans and large animals, benefitting patients and clinicians by contributing to the development of effective cell-based therapies and new physiotherapeutic rationales, which will ultimately reduce the economic and health burden caused by [organ] injury.*

*Likelihood of achieving these benefits:*
*Most of the approaches we intend to use are described in the literature. I have established a collaboration with Professor Brown's group at XXX University to learn their methodology. Approach X is novel and will require development work under this project authority as explained in Part D.*

| **TOTAL NUMBER OF WORDS (PART C):** |
|---|

**References**

| List up to 10 key references and/or regulatory guidelines supporting the need for the work and/or benefits set out above and relevant references for any specific models proposed in your programme of work.<br>. |
|---|
| C3.1 References should typically relate to:<br>• the scientific background. We would normally expect most/many of these to be published within the last few years and typically to include references from your own and other groups;<br>• if this is a replacement project licence, this should be updated to reflect developments over the past 5 years<br>• proposed models and/or methods.<br><br>C3.2 Include the regulatory basis for the work for projects involving regulatory toxicology/testing work.<br><br>C3.3 Please provide as full a reference as possible (Authors, date, title and journal and links where relevant) and ensure they are correctly referenced in the text where appropriate.<br><br>C3.4 Upload any key reviews or method papers as ASPeL Supporting Documents or as a hyperlink (please check they are still current) to assist the inspector where possible - see ASPeL Quick Start Guide for explanation of uploaded documents. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/579962/PPL-Quick-Start-Guide-Applicants.pdf You can also upload a list of your own publications or other outputs as Supporting Documents. Supporting Documents will not form part of the licence. |

## D. PROGRAMME OF WORK

The total response to this Part must not exceed 2000 words

### Purpose

A project licence may be granted for a programme of work to be carried out only for one or more of the following purposes (ASPA section 5C(3)):

**You will be required to tick each of the boxes on ASPeL which applies to your project**

| | |
|---|---|
| X* | (a) basic research; |
| | (b) translational or applied research with one of the following aims — |
| X* | (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants; |
| X* | (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants; |
| X* | (iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes. |
| | (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b); |
| | (d) protection of the natural environment in the interests of the health or welfare of man or animals; |
| | (e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work; |
| | (f) higher education or training for the acquisition, maintenance or improvement of vocational skills; |
| | (g) forensic enquiries. |

*** - these are the most common purposes for general licences.**

### Aims and objectives of the project

To assess the likely benefits of your project we need to understand what you aim to achieve, find out, establish, or produce by undertaking the proposed programme of work

To enable us to do that you should:

- Define succinctly the overall purpose or aim of your project
- Where appropriate, identify the separate key elements/questions or specific objectives that are to be addressed to achieve the overall aim

The overall aim should be specific to your project, unambiguous, realistic and achievable

D1.1 **Suggested approach** – identify the overall aim, then set clear specific objectives.

D.1.2 The **aim** should be summarised by the **title** of the programme of work.

D1.3 ***Examples of aims***

*A. To assess the efficacy of gene therapy to treat a range of lung and other human diseases.*

*B. To determine whether combinations of inhibitors of vascular endothelial growth factor (VEGF) and chemotherapy are more effective than monotherapy in preventing tumour vascularisation and tumour growth.*

*C . Identification of changes in neuronal ion channels that are associated with the development of peripheral neuropathic pain.*

*D. The overall aim of this program of work is to use animal models to assist in the discovery and development of new therapies for the treatment of respiratory diseases (such as asthma, chronic obstructive pulmonary disease and respiratory tract infections).*

*E. The aim of this project is to characterise a newly emerging disease of livestock, XYZ disease, that is spreading across Europe.*

D1.4 For larger research projects, subdivide the overall aim into a number of **objectives**. You may wish to describe these objectives as questions (see examples below). You may not be able to identify all of these objectives at the time of application. Additional objectives can be added as amendments if they fit within the overall aim.

D1.5 **Objectives** should be as SMART (specific, measurable, achievable, realistic, time-related) as possible. It should be possible to determine, in five years time, whether or not they were met, assuming all lines of enquiry are pursued.

D1.6 Taking into consideration your expected funding, staffing and other resources, what can you realistically expect to achieve? Make those the objectives in your application. You may only be able to identify realistic achievements for the next 2-3 years in a rapidly advancing field. You may find it simpler to apply for a programme of work for a shorter duration than 5 years and then apply to extend the licence authority for up to 5 years if/when you later identify additional objectives.

D1.7 Usually, general terms like “cancer”, “nervous system”, “receptors” or “pathways” are too broad without specific explanation of what you are aiming to do:

- Which types of cancer? What aspects of disease?
- What elements of the nervous system, with what effects?
- Which types of receptors involved in the control of ...?
- Pathways affecting what (patho) physiological processes?

D1.8 Try to ensure your objectives reflect the **outcomes** you want to achieve, not the

methods you will be using to achieve the outcomes (e.g. creating a new line of genetically altered mice is a *method* of achieving a scientific objective rather than the scientific outcome desired. The *outcome* could be, for example, determination of the role of gene X in controlling the response of Y lymphocytes in Z disease state which would require, along with other methods, the development and use of a genetically altered mouse strain which overexpressed X to achieve this objective).

D1.9 Include 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 the drug discovery programme example below, initial identification of molecules that interact with the target will normally be done in vitro or from a literature search.

D1.10 The objectives listed in this section should determine the benefits identified in Part C.

D1.11 ***Examples of aims and objectives:***

***Example 1: Drug discovery programme***
*The overall aim is to identify and select for further development approximately 3 potential new therapeutic agents with demonstrable efficacy in animal models of hypertension.*

*a) Which molecules interact with the target?*
*b) Which molecules are most efficacious?*
*c) Are the selected compounds sufficiently potent? And do they have a suitable PK profile?*
*d) Does the selected compound modulate blood pressure in vivo?*
*e) Does the selected compound lower blood pressure in a disease model?*

***Example 2: Fundamental research ('blue sky')***
*This project aims to define the functions of selected genes involved in obesity and metabolic diseases. Specifically we will ask:*

- *Is expression of genes affecting pathway X regulated physiologically?*
- *Does induced alteration in gene expression affect energy balance, carbohydrate & lipid metabolism or blood pressure?*
- *Can the effects of alterations in gene Z be rescued by drugs acting on its pathway or by restoration of its normal expression?*

***Example 3: Fundamental research (hypothesis-led)***
*I aim to determine how the Myc oncogenic system regulates epidermal proliferation and differentiation processes and whether the molecules in the system increase or inhibit tumour development. I will ask:*

1. *Are GA mouse lines mis-expressing candidate genes viable in vivo?*
2. *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?*
3. *Does mis-expression of candidate genes alter the expression of Myc-regulators in stem cell?*
4. *Does mis-expression of candidate genes affect proliferation and differentiation of normal skin?*
5. *How does mis-expression of candidate genes impact on skin stem cells and regeneration after injury?*

***Example 4: Translational research***

*The objectives together will enable a new disease of livestock, XYZ disease, to be characterised to inform development of potential diagnostic and control strategies. To do this the following need to be determined:*
*1. Minimum infectious dose by the expected route of infection and determination of the attack rate*
*2. Pathogenesis of the infectious organisms in pregnant and neonatal pigs*
*3. Likely mechanisms of spread between animals, including the role of asymptomatic carriers of both the target species, pigs, and other species thought to act as reservoirs of infection*

D2.1 In Part D, do not duplicate information that you have provided elsewhere (e.g. background, benefits, resources or expertise). **Avoid repetition** between the project plan, 3Rs sections and protocols; Part D should include **why** you need to undertake the regulated procedures contained in the protocols, how these will achieve the objectives and **why** you have chosen the most refined models and methods likely to meet the scientific need, while the protocols in Part E specify **what** will happen and **what** you will do to minimise suffering.

| **Explain** in Part D: | **Specify/Describe** in the Protocols |
|---|---|
| • **How** the protocols are used to achieve the aim and objectives;<br>• **Why** the particular animal models; techniques, experimental designs etc have been chosen and are most likely to achieve the scientific goals;<br>• **Why** the adverse effects are unavoidable;<br>• **Why** it is necessary to keep animals showing adverse effects; and<br>• **Why** significant adverse effects cannot be ameliorated.<br><br>• **For severe severity limit protocols :**<br>• Provide a robust justification based on scientific necessity.<br>• Show clearly in either the project plan or the 3Rs section, **how** numbers and suffering will be minimised. | • **What** type and number of animals will be used;<br>• **What** procedures will be performed.<br><br>And in the adverse effects section:<br><br>• **What** their effect on the animals will be;<br>• **What** the likely incidence is;<br>• **What** measures will be taken to minimise the incidence;<br>• **What** measures will be taken to prevent or control adverse effects; and<br>• **What** general humane endpoints will be applied to the protocol as a whole. |

**Project plan**

Provide an overview of the project:

- Explain the sequence and inter-relationship of the project's key elements or specific objectives or stages in achieving your aims
- Provide an outline of the stages of the programme of work and indicate clearly, by using the protocol numbers, how each protocol will be used to achieve your objectives
- Describe where decision points occur in the sequence of work and how subsequent steps will be affected by those decisions. Where it would aid clarity, illustrate the steps of the programme using an annotated process map or decision tree (to be uploaded in ASPeL as a licence attachment and referenced in the body of the text e.g. see Annex 1)
- Indicate how *in silico*, *in vitro* and *ex vivo* work integrates with your *in vivo* work, the relationship between each component of the project and the sequence of the work

For each specific objective or key element:

- Identify the data or products that are needed to achieve the purpose of the project. Explain how those data or products will be generated using the different sequences of procedures specified in the protocols
- Explain how you will select the most appropriate methods and models
- Provide scientific justification for the regulated procedures and resultant harms

Where animals are to be re-used:

- Explain the reason for the proposed re-use, taking account of the balance between refinement and reduction
- Identify the welfare and scientific considerations that will be used to determine suitability for re-use
- State the criteria that will be used by the veterinary surgeon to determine that animals can be kept alive and re-used, including any limitations on the period of time that the animals will be held under the supervision of the NVS/veterinary surgeon.

D3.1 **General**
This section **links your objectives to the protocols** and **explains what you want to do and why.** It contributes to the inspector's assessment of:

- whether the programme of work meets legal requirements;
- the **likelihood of success**;
- whether non-animal alternatives could be used to achieve all or part of the programme of work;
- the **harms** to the animals;
- whether the harms are scientifically justified;
- how you will be applying the **3Rs** to the techniques you will be using and whether your choice of models are most likely to yield satisfactory results.

D3.2 Throughout this document, the terms 'procedures' and 'techniques' are used interchangeably and refer to generally single events such as taking a blood sample, imaging or ovariectomy. The term 'protocol' refers to the description of one or more procedures applied to a group of animals for particular purpose – see further explanation in Part E.

D3.3 We recommend that you complete this section in two parts. First, give an overview of your scientific strategy for achieving the aim of the project. Then explain, in turn, how you will achieve each objective.

**D3.4 Scientific strategy for achieving the aim of the project:**

- 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. Use the objectives from the aims and objectives section above. Your process maps/decision trees will need to be uploaded as separate documents – as a 'Supporting Document' for draft applications and only as an 'Attachment' in the formal application.
- 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 a number of sequential stages, explain the criteria for progressing to the next stage.
- Do not include detailed descriptions of the procedures or models at this stage.
- Do not repeat information given elsewhere in this application (e.g. Part A resources and expertise, Part C background and benefits).

D3.5 **Examples** of three process maps are provided at the end of this document (Appendices 1, 2 and 3):
- ***Example A.*** The first example continues the drug discovery project described in the aims and objectives section above.
- ***Example B.*** The second example illustrates the proposed work plan for a project investigating whether candidate genes have a role in energy and/or metabolic disorders.
- ***Example C.*** The third example is the process map from a complex project seeking to determine how the Myc-oncogenic system regulates epidermal proliferation and differentiation processes and whether molecules in this system increase or inhibit tumour development.

D3.6 Note that in each case the process map is based around the **objectives** the work is aiming to answer (Does the compound have a high potency and suitable pharmacokinetic profile? Does the compound lower blood pressure in an animal model of hypertension? etc). It also shows the sequence of studies, work carried out prior to, and subsequent to, this project, the protocols used, and (in the first example) the likely number of test articles investigated at each stage during the life of the project.

D3.7 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 programme.

D3.8 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.

D3.9 **Achieving the objectives**
Explain succinctly and in turn how each objective will be addressed. 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?

D3.10 Amalgamate or cross-refer to the relevant sections where the explanation or justification given is the same for more than one objective, to avoid repetition.

D3.11 Keep this section brief – it is sufficient simply to describe the main output(s) required. A list could suffice.

D3.12 Examples of data outputs
- *Tumour development and growth (volume and number of metastatic sites)*
- *Correlated visual and electronic recordings of jaw movements*

D3.13 Examples of product outputs
- *High affinity anti-lipoprotein antibodies*
- *Tissue-specific genetically altered mice for gene inactivation or over-expression studies*

D3.14 **Explaining how each objective will be achieved:**

D3.15 Explain which models (including types of genetically altered animals if to be used) and study types have been selected and why you have chosen these in particular. Why are they the most refined (from an animal welfare point of view) but still allow you to achieve your objectives? Which others have you considered? Why can't you use animals with a lower capacity to experience pain, suffering, distress or lasting harm e.g. fish instead of mice? You may wish to put this information into the "Refinement" section below but do not repeat information. You may cross-refer between these sections.

D3.16 Explain the various sequences of procedures (both regulated and non-regulated) that will be carried out to achieve the objective. Relate these to the protocols used and make sure that the reasons for any alternative or optional steps in the protocols are clear. Note: in this section provide an *explanation* for the protocols, *not a description* of them.

D3.17 ***Illustrative example of generating outputs to achieve an objective:***
*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 diabetes generation protocol (protocol 7). We will compare the disease progression in the different genotypes by monitoring:*

- *urine glucose*
- *blood glucose*
- *body weight*
- *water consumption*
- *degree of polyuria*
- *post mortem histopathology*

*In some knockout animals we will use inducers to try rescue the diabetic phenotype. [Example of an optional step.]*
*We will generate diabetic mice by either inducing the disease chemically (STZ) or by feeding a high fat diet in order to determine the role of the gene in models of Type 1 and Type 2 diabetes. [Example of alternative steps.]*

D3.18 When you have drafted your protocols, cross-check to ensure that you have provided a scientific rationale for all the procedures for each protocol in this section. The licence authority needs to be clear as to **what** you propose to do and the inspector needs to understand **why** it is scientifically necessary.

D3.19 Try to avoid repetition. If the reason why you will use a particular procedure (e.g. blood withdrawal using minimum severity procedures) is done for the same purpose (e.g. determination of blood glucose) in each protocol, then a general statement to that effect is all that is necessary. If however you will need to obtain blood samples for different purposes and this will sometimes require larger volumes (e.g. blood glucose determination in some protocols and large volumes (up to 15% total blood volume) on a single occasion for measuring a particular metabolite) then you need to provide more information to clarify this.

D3.20 Explain what your scientific and humane endpoints will be. You need to explain the degree of pathophysiological changes that is scientifically necessary in order for you to

achieve your scientific objectives. How do these correlate to 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?

- 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.

D3.21 Explain how and when **pilot studies** (e.g. dose range finding, method validation etc) would be used. See https://www.nc3rs.org.uk/conducting-pilot-study

D3.22 Explain what checks you will do to assess the likely suitability of substances given to the particular strain/type of animal you will be using. How will you determine your dosing regimen, to make sure that adverse effects are minimised?

D3.23 Use principles, performance measures and illustrative examples wherever possible.

D3.24 Examples of principles, performance measures and experimental design principles:

D3.25 ***Principles***

- *Blood samples will be taken either by venepuncture or using a vascular cannula depending upon the number and frequency. If sampling over a longer period of time cannulae may be implanted surgically.*

- *If the genetic manipulation results in a phenotype of moderate or severe severity, or in cases of perinatal mortality, experiments will be conducted in which epidermis or tumour/cell lines are grafted directly into host mice instead of generating transgenic lines.*

D3.26 ***Performance measures***

- *Whole body irradiation will be used to suppress the immune response. The doses of radiation selected will be such as to avoid clinical signs of toxicity over the duration of the experiment.*

- *Blood samples will be taken at a frequency and volume to generate an appropriate pharmacokinetic profile. Typically 5 – 8 time points using 2-6 animals in total. Animals should only experience transient pain and no lasting harm.*

D3.27 ***Experimental design principles (either in the project plan or in the Reduction section)***

- *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 adverse effects, are observed.*

- *Unless there are good reasons otherwise, the experiments will be designed to compare dose levels and factorial experimental design will be used when applicable.*

- *If toxicity is not known the agent will be used on unoperated animals under Protocol 1 – Dose setting, at a low dose in no more than two animals initially. 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 by a similar stepped, minimal numbers approach.*

D 3.28 **Keeping alive**
If animals will be kept alive at the end of a protocol you must read and follow the Advice Note on Use, keeping alive and re-use. This also explains about continued use.
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/470008/Use__Keeping_Alive_and_Re-use_Advice_Note.pdf

D3.29 **Anaesthesia**
**Important:** Include this wording in order to explain the use of anaesthetic codes in the protocols:
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 will be indicated by using the following codes in protocols: AA (no anaesthesia); AB (general anaesthesia with recovery); AB-L (local anaesthesia); or AC (non-recovery general anaesthesia).

D3.30 If you are using neuromuscular blocking agents you will also need to add 'AD (code for under neuromuscular blockade)' and complete the section on 'Use of neuromuscular blocker' below.

D3.31 You should take the advice of your veterinary surgeon to select the most refined anaesthetic regimen for your studies that will not interfere with the scientific outcomes. The detailed outcome of that advice does not need to be included in the application

D3.32 **By the end of this section the inspector should understand why you need to use animals, how they will be used and why the harms are necessary for the science.** There should be no surprise procedures when the protocols are read.

D3.33 **Specific advice about certain types of licence:**

D3.34 ***Referral to the Animals in Science Committee (ASC)***
Some types of work will need to be referred to the ASC during the process of assessment. Para. 13.5 of the Guidance summarises this requirement. In addition some applications may be referred within ASRU for further consideration and assessment. For example, applications with protocols that have a severe severity classification (any species) and/or protocols entailing use of certain techniques such as retro-orbital blood sampling might be referred to another inspector to ensure consistency of approach.

D3.35 ***Use of animals taken from the wild***
See Advice Note 'Working with animals taken from the wild'
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf
Please see attached specific check list (Appendix 5) that you can use to help you identify all the information needed and then include it in applications for using such animals.

D3.36 ***Education and training licences***
There are special requirements for these types of licences – please contact your assigned inspector early in the process of drafting your application.

D3.37 ***Work classified as severe***

Work classified as severe will require detailed and robust explanation and a strong scientific justification. You should indicate you are aware of and have taken account of recent expert working group papers on refinements for any relevant severe disease models e.g. sepsis, arthritis or stroke.

D3.38 ***Non-schedule 1 killing techniques***

If one or more protocols require the use of non-schedule 1 killing techniques for scientific reasons, this section must explain why the purposes of the programme of work cannot be achieved using a schedule 1 method. See section6.6.2 b) of the ASPA Guidance for further details.

**THE 3Rs**

The Secretary of State must assess how the programme of work complies with the principles of replacement, reduction and refinement. These principles are described in section 2A(2) of the Act.

D4.1 Project proposals have to balance the greatest likelihood of generating satisfactory results, using the least number of animals and causing the least severe adverse effects.

D4.2 **Note: There is no need to repeat information already provided in the project plan above; the information provided in the 3Rs sections should complement that provided in the project plan.**

**Replacement**

- Why is it not possible to achieve the objectives of your project without using animals?
- What alternatives have you considered and why are they not suitable? What alternatives will be used in achieving your objectives?
- What, if any, *in silico*, *in vitro* or *ex vivo* techniques will you use and how will they integrate into this project?
  (if not already covered in the project plan)

D5.1 Ensure you have provided information to address each of the prompts above.

D5.2 Research all possible alternatives for your programme of work. Your AWERB and Named Information Officer can help you find suitable databases and websites. For example, have you fully considered practicable alternative approaches such as:

- computer modelling;
- in vitro methods such as cell culture, organs on a chip, organoids;
- non-protected species such as fruit flies or nematodes;
- human data?

D5.3 Describe the steps you have taken to research non-animal alternatives. In some cases replacement methods may be able to answer some of your scientific questions and you can describe any limitations here. You should include relevant research that indicates all or some of the models you wish to use could be replaced and explain why your research is an exception. For example if you are intending to raise antibodies using animals, consider why the use of non-animal phage display techniques cannot be used instead (e.g. Gray et al (2016) Animal-Friendly Affinity Reagents: Replacing the Needless in the Haystack. *Trends in Biotech* 34 (12) 960-969).

D5.4 SyRF is a free online platform for researchers to perform a systematic review and meta-analysis of animal studies. The platform was developed by CAMARADES and groupfunded by NC3RS: https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf

D5.5 Explain if you think you will be able to replace any part or all of your proposed animal use during the course of this programme of work.

**Reduction**

- What measures have been or will be taken to ensure that the minimum number of animals will be used in this project?
- Explain the principles of experimental design you will use and any sources of advice you will consult e.g. on statistics, experimental design
- How will you control sources of variability?

D6.1 See prompts in box above.

D6.2 Where appropriate, outline the **principles** of experimental design you will use at each stage of the work, indicating:

- how the different experimental groups (controls, dose levels, satellites etc) will be chosen;
- how control groups are used. Provide a robust scientific justification for the use of sham surgical controls;
- how you will maximise the data output from the animals you use;
- how likely variability will be determined and minimised;
- how group sizes will be set. Does data exist from previous work?;
- how studies are randomised and blinded;
- when and how pilot studies will be used;
- how comparisons will be made between groups or experimental situations;
- how data are analysed to ensure the maximum efficiency of animal use.

D6.3 Considerations:

- what measures have been or will be taken to minimise and control the sources of variability within your project and its component procedures?
- what experimental design principles will be followed and what sources of specialist advice will you consult e.g. on statistics?
- what other measures have been, or will be, taken to maximise success and minimise unnecessary use of animals?
- Include other measures that you will use to reduce the number of animals used such as use of ex vivo material from tissue banks or surplus stock

D6.4 The experimental principles should achieve reliable results, avoid unnecessarily repeating experiments and allow decision making. Considerations are not simply about using few animals; sufficient animals need to be used to achieve the stated objectives. An inadequate group size in experiments leading to unsatisfactory results is a waste of animals. Small groups will suffice for pilot studies. See: https://www.nc3rs.org.uk/conducting-**pilot**-study

D6.5 Explain how you will ensure any breeding of GA lines is as efficient as possible and

how you will ensure that genetic integrity is maintained. You should clarify whether you will be using gene editing technologies such as CRISPR to generate any new GA lines and the scientific or 3Rs rationale for not doing so, if that is the case.

D6.6 You should confirm that you will be conducting and recording your experiments to be able to publish your results following the ARRIVE guidelines (or explain why not) [https://www.nc3rs.org.uk/arrive-guidelines] and will use randomisation, blinding etc. where appropriate so as to minimise biases.

D6.7 You should consider whether factorial designs are suitable. Power analysis is often useful in determining group size, but is not always applicable. Specialist advice on experimental design and statistical analysis of results is usually widely available at establishments.

D6.8 The estimated number of animals is a key part of the inspector's harm-benefit analysis (and the rationale for this must be clear from section D): https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/487914/Harm_Benefit_Analysis__2_.pdf

D6.9 Sources of information include:

- NC3rs experimental design assistant https://www.nc3rs.org.uk/experimental-design-assistant-eda
- 'Beware of power failure': Button et al. (2013) Nat Neurosci 14, 365-376

D6.10 ***Example 1***

*Advice on the proposed experimental designs and methods of analysis of the results will be taken from the Statistical Services Unit. 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 6-8 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. Furthermore, as part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material; and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated). We will make appropriate arrangements to randomly assign animals to experimental groups and blind studies and will plan and conduct studies to enable them to be published according to the ARRIVE guidelines.*

D6.11 ***Example 2***

*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). This part of the programme will be implemented by the Transgenics Officer, who has the expertise required to deliver satisfactory results (i.e. healthy*

*animals of the desired genotype).*

*We now use CRISPR/Cas9a which should bring benefit both to the reduction of the numbers of animals used and refinement in the ability to create genetically altered mice of higher quality.*

*Efficient colony management ensures that only colonies that are actively being used are mated and produce animals. Those that are no longer required are cryopreserved and closed at the earliest opportunity. We now archive sperm as the main method of cryopreservation which reduces the number of animals required to secure a line. We will use good practice in experimental planning, including statistics such as power and resource equations and breeding calculations using current breeding figures to predict the number of matings required for experimental cohorts.*

*From our previous experience we expect xx animals will be required to produce a new line, and yy/year to maintain an established line: nevertheless, we will measure production and breeding performance and ensure the minimum numbers of animals are used in the programme.*

D 6.12 ***Example 3***

*When comparing hypertension in SHR rats during the proposed interventions, it is vital to obtain clear data on the evolution of the hypertension (which starts at around 6 weeks old) as well as on its final degree (by around 12 weeks). This can be obtained far more efficiently in animals with transducers chronically implanted into their descending aorta. Over 90% of such implants permit continuous recording for 3 months. Without such recording acute experiments would be required at weekly intervals from ages 5-14 weeks. The use of transducers, therefore, reduces animal use by around 90% and in addition provides paired longitudinal recordings that greatly strengthen the statistical analysis. The cost to the animals is the surgery and post-surgical recovery of those animals used, but with such simple surgery and minimal clinical after-effects this is at the low end of moderate severity and represents a valuable reduction in overall harms.*

**Refinement**

- Explain your choice of animals, model(s) and method(s). Explain why they are the most refined for the intended purpose
- How will you minimise animal suffering while carrying out your work?
- Provide specific justification for any protocols categorised as 'severe'

D7.1 See prompts in box above. You don't need to repeat information that is already in the project plan.

D7.2 **Surgery**

We expect all recovery and long-term non-recovery surgery will be done aseptically (see the Appendix 4 HO Minimum Standards for Aseptic Surgery. See also other guidelines e.g. LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery http://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf

D7.3 Can you perform procedures completely under non-recovery anaesthesia to answer some of your research questions?

D7.4 **Analgesia**

We also expect that peri-operative analgesia will be given and maintained after surgery for as long as is necessary to alleviate pain. Describe how you will monitor for pain and provide appropriate levels of analgesia – e.g. use of grimace scale, use of self-

administered analgesics.

D7.5 If you will not be using analgesia to control pain after surgery or other painful procedures which would normally require analgesia, you *must* make this explicit and explain why, scientifically, you cannot use analgesia. Also you must explain what other measures you will use to mitigate the effects of the procedures.

D7.6 **Choice of models and animals**
If not already covered in the project plan, explain here and cross reference your choice of animals, model(s) and method(s), showing, as relevant to your programme of work:

- why the type of animal, the model(s) and methods selected are together likely to result in the least pain, suffering distress or lasting harm to the animals involved to achieve the scientific benefit;
- what other options are available;
- what principles are followed when selecting between options of different severity;
- why further reductions in the intensity or duration of adverse effects cannot be achieved;
- why you cannot further reduce the capacity to suffer, for example using anaesthesia, analgesia or using animals at a different life stage;
- how you intend to refine the studies during the course of this project;
- how you will ensure that animals are maintained in the best physiological state;
- why it is not possible to conduct your studies using earlier end points.

D7.7 Include specific justification for any protocols that may be classified as severe. What strategies will you use to reduce severity, for example a frequent monitoring regime, if necessary throughout the night, use of score sheets, use of biomarkers to establish earlier endpoints at which you can gain satisfactory data? You should consult published guidelines to ensure refinement of particular disease modles, for example IMPROVE guidelines for stroke models: https://www.nc3rs.org.uk/news/improve-ing-animal-welfare-experimental-stroke-research

D7.8 Include specific justification for withholding any treatments or standard husbandry practices that may impact on animal welfare.

D7.9 There are published guidelines to assist with planning animal research and testing, such as the PREPARE guidelines:
http://journals.sagepub.com/doi/full/10.1177/0023677217724823
Further information about the PREPARE guidelines can be found here:
https://norecopa.no/prepare

D7.10 *Example*
*We intend to use three models of peripheral neuropathic pain:*
*• chronic constriction injury (CCI or Bennett model) - four chromic gut sutures are loosely tied around the sciatic nerve;*
*• partial sciatic nerve ligation (PSL or Seltzer model) - ligation of the ipsilateral sciatic nerve at high thigh level so that 1/3 to 1/2 the thickness of the sciatic nerve is trapped in the ligature;*
*• L5/L6 (rat) or L4/L5 (mouse) spinal nerve ligation model (SNL or Kim & Chung model) - L5 and L6 (rat) or L4 and L5 (mouse) are unilaterally and tightly ligated distal to the dorsal root ganglia.*

*Each of these models has slightly different characteristic effects:*

| *Model* | *Spontaneous pain* | *Autotomy* | *Mechanical allodynia* | *Cold allodynia* | *Thermal hyperalgesia* | *Mechanical hyperalgesia* |
|---|---|---|---|---|---|---|
| *CCI* | *+++* | *+* | *+* | *+++* | *+* | *+* |
| *PSL* | *++* | *±* | *++* | *++* | *+* | *+* |
| *SNL* | *+* | *-* | *+++* | *+* | *+* | *+* |

*Behavioural signs of spontaneous pain include guarding, excessive licking and lameness in the ipsilateral hind paw. Hyperalgesia and allodynia are detectable. 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.*

*We do not intend to use other standard models of peripheral nerve injury, such as the neuroma model, which are either more severe or less severe but not relevant to the objectives of this project. All animals,may experience some post-operative pain or discomfort because of the surgery.*

*There is some evidence that NSAIDs interfere with the induction and development of pain responses in nerve injury models (Zhao et al (2000)). However we intend to run a pilot study in a small group of animals, in which groups with and without post-operative analgesia using other analgesic agents will be compared to determine if there is an analgesic regime that provides satisfactory scientific outcomes. Soft bedding, wet mash, chew blocks and supplementary heat will be provided to help mitigate the pain.*

*The precise adverse effects of the genetic alterations are not known. Some lines may be embryonic lethal or lethal before adulthood (eg Nav1.7), and such lines will be made as conditional knockouts or be maintained as heterozygotes. We intend to use reporter animals, eg to aid electrode targeting. We also intend to make animals with changes in ion channel trafficking proteins. For each line, a detailed phenotypic assessment will be made, for inclusion in a mouse passport.*

**Origin(s)**

*Annex VI* of the Animals Directive requires information on the origin(s) of animals to be used

| List the likely origin(s) of animals to be used in this project (e.g. UK, EU, or Non- EU Establishments). |
|---|
| D8.1 The information here contributes to the harm benefit analysis, for example potential risks to welfare from long journeys.<br><br>D8.2 This must be filled in. 'N/A' is not suitable.<br><br>D8.3 If the origin of the animals needs to change during the lifetime of the project (e.g. originally all animals were to be sourced from the UK but they are now no longer available) then this section will need to be amended if you need to source animals from abroad.<br><br>D8.4 State if all species to be used that are listed in Schedule 2 of the Act will be purpose-bred. If not, explain here why, scientifically, purpose-bred animals cannot be used. |

## SPECIAL CONSIDERATIONS

### Cats, dogs, primates and equidae:

Under the Animals (Scientific Procedures) Act 1986 section 5C(4), the Secretary of State must verify that additional conditions are met before authorising the use of cats, dogs, primates or equidae

| If you intend using cats, dogs, primates or equidae, explain why no other species is either suitable for the purpose or practicably available |
|---|
| D9.1 **Cats, dogs and equidae**<br><br>D9.2 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:<br>(a) only by the use of cats, dogs or equidae; or<br>(b) only by the use of cats, dogs or equidae and other animals which it is not practicable to obtain.<br><br>D9.3 The availability of background data or 'the usual species of choice' are not adequate justifications in themselves.<br><br>D9.4 **Non-endangered primates**<br><br>D9.5 Explain why the purposes of the programme of work cannot be achieved by using species that are not primates.<br><br>D9.6 Explain why the project is:<br>• 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<br>• 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<br>• basic research; or<br>• research aimed at preserving the species of animal subjected to regulated procedures.<br><br>D9.7 Note that applications proposing the use of cats, dogs, equidae or non-human primates in procedures classified as severe will be referred to the Animals in Science Committee (ASC) for additional advice. |

**Endangered species**

Under the Animals (Scientific Procedures) Act 1986 section 5C(4), the Secretary of State must verify that additional conditions are met before authorising the use of endangered species

| If you intend using an endangered species, explain why the purpose of the programme cannot be achieved without their use |
| --- |
| D10.1 Applications for using endangered species - those listed in Annex A to the Council Regulation EC 338/97 which are not bred in captivity - will need to be referred to the ASC.<br><br>D10.2 **Endangered primates**<br><br>D10.3 Explain why the purposes of the programme of work cannot be achieved by using species that are not endangered primates.<br><br>D10.4 Explain why the project is:<br>• 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<br>• 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<br>• research aimed at preserving the species of animal subjected to regulated procedures.<br><br>D10.5 **Other endangered species**<br><br>D10.6 Explain why the purposes of the programme of work cannot be achieved by using species that are not listed in Annex A to the Council Regulation EC 338/97 (EU Wildlife Trade Regulations).<br><br>D10.7 Explain how the project is for:<br>• translational or applied research for the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants; or<br>• the development, manufacture or testing of the quality, effectiveness and safety of drugs, feed-stuffs or any other substances or products for the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants or assessment, detection, regulation or modification of physiological conditions in man, animals or plants or the improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes; or<br>• research aimed at the preservation of the species. |

**Animals taken from the wild**
Under the Animals (Scientific Procedures) Act 1986 Schedule 2C Part 3, no protected animal taken from the wild may be used unless the Secretary of State considers an exception justified.

If you intend using wild-caught animals, explain:

- Why your aims and objectives could not be achieved without their use
- How you will minimise harms arising during their capture and release

D11.1 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).

D11.2 You should read the Advice Note 'Working with animals taken from the wild' https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf to ensure you provide the necessary information. See also attached check list at Appendix 5.

D11.3 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 a project licence application to ensure these are met. You cannot use stray animals and there are restrictions on the use of other types of animals, such as feral individuals.

D11.4 Explain why the aims of the project cannot be achieved by using animals which have not been taken from the wild.
*Example: Wild rats are required as we are investigating the incidence of a rodenticide resistance gene in wild populations.*

D11.5 Explain how the harms of the capture method being used will be minimised and how the competence of those catching the animals will be assessed.
*Example: Wild birds will be captured by people with a current BTO ringers' permit allowing unsupervised capture of the relevant species.*

D11.6 Explain how any animals found to be suffering ill health or injury at the time of capture will be examined and whether they will be treated or killed to minimise suffering.

D11.7 Explain how the health of the animals will be assessed in the course of and at the end of the series of regulated procedures and if the animals will be set free to the wild.

D11.8 The Advice Notes 'Re-homing and setting free of animals' and 'Use, keeping alive and re-use' also provide helpful information for animals taken from the wild. Hyperlinks are provided in the Protocols section.

D11.9 If you intend to use animals at a 'place other than a licensed establishment' (POLE), make sure you complete the relevant section in Part B.

D11.10 Note that applications that propose the use of wild-caught primates will be referred to

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| the ASC for additional advice. |

**Marmosets**

Under the Animals (Scientific Procedures) Act 1986 Schedule 2C, Part 3, marmosets that are not offspring of animals bred in captivity, or have not been obtained from a self-sustaining colony, must not be used unless the Secretary of State considers an exception justified.

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| If you intend using marmosets other than animals that are the offspring of marmosets which have been bred in captivity, or that have been obtained from a self-sustaining colony, explain why the purposes of the programme of work specified in the licence could not be achieved without their use. |
| D12.1 A self-sustaining colony is one where:<br>• the colony is kept in captivity in a way that ensures the animals are accustomed to humans;<br>• the colony consists only of animals that have been bred in captivity; and<br>• the colony is sustained only by animals being bred within the colony or animals that are sourced from other self-sustaining colonies. |

**Feral animals of a domestic species**

Under the Animals (Scientific Procedures) Act 1986 Schedule 2C, Part 3, no feral animal of a domestic species may be used unless the Secretary of State considers an exception justified.

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| If you intend using feral animals of a domestic species, explain why the purposes of the programme of work specified in the licence could not be achieved without their use. Note that authority cannot be given for the use of stray animals. |
| D13.1 Explain<br>• why no other purpose-bred animal is suitable for the purpose of the programme of work or is practicably available; and<br>• how the use of feral animals is essential to protect the health or welfare of that species or to avoid a serious threat to human or animal health or the environment.<br>**These are the only permitted purposes for using feral animals.**<br>D13.3 As such animals will generally have been taken, at some point, from the wild you should read the Advice Note 'Working with animals taken from the wild' to ensure you provide the necessary information.<br>https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf<br>D13.4 See also the attached check list at Appendix 5. |

**USE OF NEUROMUSCULAR BLOCKING AGENTS**

Under the Animals (Scientific Procedures) Act 1986 section 17, neuromuscular blocking agents may only be used if expressly authorised by the personal and project licence. The

Secretary of State must not grant a project licence that authorises the use of a neuromuscular blocking agent unless the Secretary of State is satisfied, on the basis of a scientific justification, that the purposes of the programme of work specified in the licence cannot be achieved without the use of such an agent.

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| If you intend using neuromuscular blocking agents in any part of this project:<br>• Give details of how they will be used including the anaesthetic and analgesic regimen<br>• Detail the methods available to ventilate the lungs and the methods used to assist in monitoring the depth of anaesthesia<br>• Explain why the purposes of the programme of work specified in the licence cannot be achieved without the use of such an agent. |
| D14.1 See Appendix H of the Guidance and the prompts above.<br><br>D14.2 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.<br><br>D14.3 Ensure that the scientific need to use them is explained clearly in the project plan.<br><br>D14.4 You need to provide evidence of existing competence of staff or how such competence will be obtained. |

**TRANSFER OF ANIMALS**

**Continuation of work**

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|---|
| If you are seeking authority in this application to continue work under one or more current ASPA project licences, provide the number of the relevant expiring project licence(s) and expiry date(s). |
| D15.1 Don’t forget to complete this section to authorise continued use from a current, expiring licence i.e. transfer of animals already undergoing regulated procedures in a protocol on the expiring licence, to a similar one on this project. |

## E. PROTOCOLS

E1.1 This is a key section for the inspectors' harm benefit assessment. It specifies the regulated procedures that are authorised and forms part of the licence, and specifies the adverse effects and control measures, including humane endpoints, which you are asking to be authorised. These must be clear and explicit so that all the harms animals may experience can be understood and assessed by the inspector.

E1.2 Protocols are the principal source of information for personal licensees and animal care staff and must therefore be clear and comprehensive.

E1.3 The term "protocol" is used to describe a single regulated procedure or a series of regulated procedures applied for a particular experimental or other scientific purpose to a protected animal. In most cases a protocol will involve all regulated procedures applied to the animal until the animal is killed or released from the controls of ASPA. Depending on the complexity of your work you may need one or several protocols.

E1.4 Protocols must specify **what** type and number of animals are proposed to be used and **what** procedures will be performed. In the adverse effects section you must specify **what** the effect of the regulated procedures on the animal will be, **what** measures will be taken to prevent or control the adverse effects, **what** humane endpoints will be applied and **what** the fate of the animal will be.

- Each protocol should cover one complete sequence of procedures carried out on an animal from start to finish of the experiment, study or production process where possible.
- Similar sequences of procedures with similar adverse effects should be grouped together in a single protocol.
- Alternative or optional steps should be identified.
- Refer to Home Office guidance/advice on use, re-use and continued use, re-homing and setting free, and use of wild-caught animals.
  https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/470008/Use__Keeping_Alive_and_Re-use_Advice_Note.pdf
  https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/470146/Advice_Note_Rehoming_setting_free.pdf
  https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf

  and Section 5.12 and Appendix G in:
  https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291350/Guidance_on_the_Operation_of_ASPA.pdf

### E1.5 How to decide when you need one or more protocols:

E1.6 In general, a **larger number of simpler protocols** provide greater clarity as to the harms that the animals will experience, rather than a small number of large, all-encompassing protocols with lots of optional steps.

E1.7 Usually, all animals used for a single study will be used on the same protocol; conversely a protocol might authorise a number of different studies. Draft different protocols if there are:

- significantly different adverse effects;

- significantly different humane endpoints;
- different life stages which have different capacities to suffer.

E1.8 **Very different species** will usually need to be on different protocols e.g. mice and fish; chickens and cattle etc. if this aids clarity of monitoring and control of adverse effects.

E1.9 **Model development and validation vs. model use**. Consider separate protocols if the monitoring, control and humane endpoints are different. It is expected that endpoints identified during model development would inform on the adverse effects for model use e.g. endpoints developed during creation of a disease model would inform on earlier or more refined humane endpoints when the disease model is used to evaluate efficacy of a test drug.

E1.10 Where **different methods** may be used **to induce the same disease condition** (and adverse effects are similar in degree), these can be included within the same protocol. For example: induction of diabetes using genetically altered mouse models (spontaneously diabetic); diet-induced or streptozotocin-induced diabetes.

E1.11 A protocol can be used to address more than one objective.

E1.12 In each protocol, details of any planned use of anaesthesia, analgesia and other pain relieving methods must be included. Use of anaesthesia in conjunction with other procedures will generally be specified as anaesthetic coding and use of pain relieving methods will generally be specified in the 'adverse effects' section.

E1.13 **For breeding and maintenance protocols for genetically altered mice,** use the example protocols available on https://www.gov.uk/guidance/research-and-testing-using-animals

*The summary table will be created automatically on ASPeL when you complete the protocols. Please copy the section below for additional protocols.*

| | |
|---|---|
| **PROTOCOL NUMBER:** | |
| **Title:** | |
| **Severity category:** | E2.1 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.<br><br>E2.2 Licences with protocols classified as severe will be subject to Retrospective Assessment. See section 5.17 of the Home Office Guidance.<br><br>E2.3 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 end-points. |

| | |
|---|---|
| | E2.4 Refer to section 5.7.3 and Appendix G of the Home Office Guidance for more information about severity classification.<br><br>E2.5 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 it. |
| **Species of animals:** | E3.1 Use of NHPs should be described on a separate protocol from other species.<br><br>E3.2 Where the drop down list on ASPeL is not specific enough e.g. birds, select 'other' from the drop down list and provide an appropriate description e.g. chickens, turkeys, ducks. |
| **Are some of these animals genetically altered: yes/no** | E 4.1 Ensure you have explained this in the project plan |
| **Estimated numbers over the duration of the project** | E5.2 Give a **realistic** estimate of the number of animals to be used **in this protocol** over the (5 year) lifespan of the project.<br><br>E5.1 The estimated number should be the number of **uses** – important if it is likely that animals may be re-used on this protocol.<br><br>E5.3 The basis for the estimates should have been **explained in the plan of work**.<br><br>E5.4 Monitor the figures regularly and seek amendment of the licence if numbers are likely to be exceeded by a significant degree (or at all in the case of specially protected species). See section 5.26 of the Home Office Guidance for definition of 'significant degree'. https://www.gov.uk/government/publications/operation-of-aspa |
| **Life stage of the animals** | **E6.1 You must include the life stage.**<br><br>E6.2 Indicate stage of development if immature forms (larvae, embryos or foetuses) or neonates will be used. Explain the lifestages used in the plan.<br><br>E6.3 Otherwise state 'adult'. |

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the proposed use now represents 'continued-use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and other advice on use, re-use and continued use.
.

E7.1 See the published Advice Note on Use, keeping alive and re-use: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/470008/Use__Keeping_Alive_and_Re-use_Advice_Note.pdf.

E7.2 **Continued use:**

E7.3 Avoid specific reference to other projects or establishments when requesting continued use because when those project licences expire or establishments change, authority will terminate.

E7.4 Remember to refer to protocols on this licence from which there is continued use of animals.

E7.5 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. In such cases the use of the same animal for the subsequent procedures is scientifically necessary and is termed '**continued use'**. 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.

E7.6 Avoid structuring your protocols to include other continued use as far as possible. In general, the protocols should not be drafted so that animals are used on several protocols to complete one experiment.

E7.7 Example – continued use of genetically altered animals

*Genetically altered animals for use in this protocol may be obtained from:*

*Protocol 4 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.*

E7.8 The import of genetically altered schedule 2 species does not require specific authorisation as continued use.

E7.9 **Re-use:**

E7.10 It is important to distinguish **use (**including **continued use)** of an animal, from **re-use.**

E7.11 See the published Advice Note on Use, keeping alive and re-use.

E7.12 ***Example 1 – Re-use of wild-type, genotyped animals***

*This licence Protocol X (Breeding and maintenance of genetically altered animals) – only wild-type animals following genotyping.*

E7.13 ***Example 2 – Re-use of animals kept alive under the supervision of the veterinary surgeon***

*Any animal that has completed a series of procedures under the authority of another project licence with primary availability at [place], and has been kept alive under the supervision of the NVS, may be re-used in this procedure in accordance with the criteria specified in the project plan and with ASPA section 14.*

**Protocol steps:**

- List numbered and broadly chronological steps starting with the first preparative step and ending with the death of the animal or the last regulated procedure in the experiment
- It is accepted that the order of steps may be varied according to scientific need
- Protocols may contain optional steps, but should include at least one step which will always be undertaken. (How you will use optional steps must be clearly explained in the programme of work)
- List alternative techniques e.g. dosing routes, as lists for clarity
- Indicate where one or more steps may be repeated within an experiment, e.g. a cross-over study
- Use anaesthetic codes but remember that administration of anaesthetics (and analgesics) are themselves regulated procedures and should, therefore, be specified
- Do not include technical detail in the description of procedures. Instead use principles & performance measures, e.g. 'light emitting substances' instead of 'luciferin'
- Specify limits and controls **only** where they impact directly on animal welfare, e.g. 'intramuscular injection (max volume 0.1ml, no more than two injections at 48 hr interval)'. In all other cases use performance criteria
- If appropriate indicate the method of killing, i.e. Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC)

**E8.1 Read the prompts above.**

E8.2 **Description of Procedures:**

E8.3 Refer to the example at the end of this section. This should clarify the meaning of 'step' below.

E8.4 Write protocols in **numbered and broadly chronological steps** starting with the first preparative step and ending with the death of the animal or the last regulated procedure in the experiment. A 'step' is a single procedure or a combination of procedures to achieve an outcome during the course of a series of procedures, for example administration of test compounds using a variety of alternative dosing routes, or imaging under general anaesthesia with associated use of contrast agents. Indicate if the order of steps will vary according to scientific need.

E8.5 Protocols may contain **optional steps**, but should include at least one step that will always be undertaken. How you will use alternative or optional steps should be clearly described in the plan of work, as a summary in the protocol or by using a flowchart showing the steps animals could experience. Make sure that control groups are catered for.

E8.6 Mandatory and mutually exclusive steps (either/or) should be clearly indicated. Steps never applicable to certain animals e.g. neonates or female animals etc, should also be clearly indicated.

E8.7 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. Competence is assumed and project licence standard condition 4 requires the 3Rs to be applied in all cases (see Appendix C of

The Guidance https://www.gov.uk/government/publications/operation-of-aspa).

E8.8 Indicate where one or more steps may be **repeated** within an experiment.

E8.9 Use **anaesthetic codes**, AA, AB, AB-L or AC as appropriate to indicate when a procedure is being done under anaesthesia; or AD for procedures undertaken under neuromuscular blockade. When administration of analgesia or anaesthesia (e.g. for restraint) is the only regulated procedure associated with an experimental step, then this must be included as a separate step. [NOTE: you need to include text explaining the meaning of these codes in the Project Plan section – see D 3.28]

E8.10 List **alternative dosing or sampling routes** – see example below.

E8.11 **Be as specific as possible** but without reducing your flexibility to change the way you work where this will not result in increased severity. You may use **performance measures or technical specifications**, for example: instead of 'injection of substances' give the class or type of substance, for example, 'antibiotics' or 'receptor agonists and antagonists'; however, you do not necessarily need to list every 'antibiotic' or 'receptor agonist or antagonist' unless they cause specific adverse effects. For example 'a non-ulcerative adjuvant' might include many types of adjuvant but if you are using Complete Freunds Adjuvant you should specify this because it can result in significant adverse effects.

E8.12 **Specify limits where they impact directly on animal welfare**. You can provide technical limits specifying maximum dose or blood sample volumes (in ml/kg), maximum number of procedures, minimum intervals etc. Alternatively you could use performance measures and say that the frequency of {specify type} injections will be such that animals fully recover between injections and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect from repeated injections. The latter approach provides more flexibility for regimes to be altered without affecting the severity of the procedures but can lead to lack of clarity, for example when deciding an animal has fully recovered. Project licence standard condition 4, of course, requires all licensees to cause the minimum harm to achieve the desired outcome.

E8.13 **Dosing & sampling**: If you intend to use injection volumes that are greater than those considered in standard published guidelines (e.g. LASA guidelines, Diehl et al (2001) *Appl Toxicol* 21, 15-23) to result in no more than mild severity you need to explain in the plan of work, why scientifically this is needed. This also applies if you want to withdraw blood in greater volumes than normally accepted limits that result in mild severity and no lasting harm. These are: no more than 10% total blood volume (TBV) at any one time and no more than 15% TBV in any 28 day period.

E8.14 Be clear if animals will be **set free to the wild during the course of procedures** (see Advice Note 'Working with wild animals').

E8.15 Be clear about **control groups**, including sham surgical controls, - which steps will they go through?

E8.16 Procedures below the threshold for regulation only need to be mentioned in the protocol where there is an expectation that they might **cumulatively** result in above-threshold levels of suffering. A series of non-regulated procedures may together cause

suffering that crosses the lower threshold, for example repeated short periods of separation from cage-mates or repeated short periods of food restriction. Such situations should be discussed with your assigned inspector. Non-regulated procedures will usually be mentioned in the project plan in Part D as part of the explanation for how the objectives will be achieved.

E8.17 **The use of appendices/attachments:**

E8.18 **Establishment guideline documents** should not normally be supplied as Attachments in ASPeL because relevant information that specifies procedures, controls, monitoring, endpoints in protocols should be included in all relevant protocols.

E8.19 However, if the same information relates to a number of protocols (e.g. 3 or more) then it may simplify the application and licence if this is uploaded as an Attachment, and appropriately referenced in the relevant protocols, so that animal care staff and licensees know where to find all the information relevant to carrying out specific procedures. Such Attachments form part of the licence and so any changes can only be made by formal amendment to the licence.

E8.20 An Attachment can also be helpful to provide detail about certain procedures to streamline protocols, e.g. behavioural tests or complex surgeries.

E8 21 **External published guidelines** should normally be considered when determining procedural steps and end points (for example LASA guidelines) **and should be referenced**. They do not need to be uploaded onto ASPeL.

E8.22 *Example – Steps in the protocol*

*Title: Skin carcinogenesis*

1. *(Optional and at any stage) Blood samples may be taken from a superficial vessel (AA).*

2. *(Optional and at any stage) Skin biopsy up to 3 mm diameter may be taken (AB). No site will be biopsied more than once with a minimum of two weeks between biopsies; no animal will be biopsied more than 4 times during its life.*

3. *(At any stage) Immunotherapeutic substances will be administered alone or in combination, continuously or intermittently by one or more of the following routes:*
    a) *in diet or drinking water (AA)*
    b) *subcutaneous (AA)*
    c) *intraperitoneal (AA)*
    d) *implantation of a slow release pellet subcutaneously on one occasion (AB)*
    e) *topical application (AA)*

4. *Tumour development will be induced by either:*
    a) *topical application of a substance (AA); or*
    b) *UVB exposure (AA)*

*5. (Optional) Some of the animals will be used in non-invasive imaging studies (e.g. NMR, ultrasound, PET, PET-CT, CT) (AA/AB).*
- *Animals may receive a single injection of contrast agent prior to or during image acquisition by the intra-peritoneal or intravenous route (AA/AB).*

- *Mice will be exposed to imaging sessions typically no more than two times per week (AB) and for no more than 5 weeks (maximum 10 imaging sessions).*

6. *Terminal studies and killing:*
   - *Killing by a Schedule 1 method*
   - *Or exsanguination (AC) followed by killing by a Schedule 1 method*
   - *Or perfusion fixation (AC)*

**Fate of animals <u>not</u> killed at the end of the protocol**

Indicate the proposed fate of animals which are not killed at the end of the protocol.

☐ **Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.

☐ **Kept alive at the licensed establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.

☐ **Discharge from the controls of the Act by setting free to the wild or by re-homing.** Specify below the particular circumstances when animals may be set free to the wild or re-homed and detail how the qualifying criteria set out in section 17A(3) & (4) will be met.

E9.1 **If you check the box 'Continued use ...':** you need to provide information about the continued use.

E9.2 Example text to permit the transfer of genetically altered animals for continued use in another protocol or another project licence:
*Following any identification of genetic status, genetically altered animals produced under the authority of this protocol and not used in other regulated procedures in this protocol may be supplied to other protocols in this project or to other projects with authority to use genetically altered animals of this type.*

E9.3 Example text to permit transfer of surgically prepared animals for continued use in another protocol or another project licence:
*Following full recovery from surgery, animals with a surgically implanted jugular catheter may be supplied to other protocols in this project or to other projects with authority to use surgically prepared animals of this type.*

E9.4 **If you check the box 'Kept alive at the licensed establishment':** you need to explain how you will determine that animals are not suffering and not likely to suffer as a result of the regulated procedures undertaken on this protocol – see the published Advice Note on Use, keeping alive and re-use and information provided in section 6 of the 'Project Plan' above.

E9.5 ***Example text for animals kept alive 1:***
*Animals that have suffered no more than mildly during the course of procedures and which are not suffering or likely to suffer as a result may be kept alive in accordance with Standard Condition 11.*

E9.6 ***Example text for animals kept alive 2:***
*Animals that have suffered no more than mildly during the course of procedures , apart from the expected recovery from surgery to implant telemeters, and which are not suffering or likely to suffer as a result may be kept alive in accordance with Standard Condition 11.*

E9.7 **If you check the box 'Discharge from the controls of the Act by setting free to the wild or by re-homing'** you will need to explain how you will meet the requirements for re-homing or setting free to the wild at the end of procedures. See the published Advice Note on Re-homing and setting free for information and examples https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/470146/Advice_Note_Rehoming_setting_free.pdf .

**Expected adverse effects, refinement controls and humane end-points:**

For the series of regulated procedures described above, both as a whole and as component steps:
Describe the likely adverse effect(s) and the expected incidence in the different animals used

- Explain how animals will be monitored for the onset or development of adverse effects
- Set out the refinement measures and other controls you will adopt to prevent adverse effects from occurring or to minimise their severity
- In all cases specify practicable and realistic humane end-points

Do not list uncommon or unlikely adverse effects or effects from procedures that cause no more than transient discomfort and no lasting harm, for example intravenous injection of an innocuous substance of small volume.

E10.1 **Also see text above.**

E10.2 **General**

E10.3 Start by providing a summary of the overall severity and include an approximate proportion of those you expect to suffer most and a general humane endpoint.

E10.4 ***Example of a summary:***
*Approximately 75% of animals are likely to experience moderate levels of severity. This is because they will undergo surgery to occlude one ureter to induce inflammation in the associated kidney, repeated individual 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.*

*Humane end-points will be determined on the basis of adverse clinical signs. 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 or develops diarrhoea or dyspnoea will be killed.*

E10.5 For each step in the protocol, discuss the adverse effects that are expected. We do not need to know about adverse effects resulting from minimal impact techniques, for example, transient pain from subcutaneous injections with innocuous materials, whether expected, uncommon or unlikely. However we do need to know what you will do should expected, uncommon or unlikely adverse effects that have significant impact on animal welfare occur. For example in the case of surgical complications following nephrectomy in mice, animals would be killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than minor interventions (see example below).

E10.6 **Step by step consideration:**

E10.7 Include the following:

- expected clinical signs and incidence;
- measures to control severity and incidence;
- humane endpoints.

E10.8 You can use general terminology to describe the incidence of effects: rare (<1%), uncommon (1-5%), common (5-10%), usual, all.

E10.9 Provide more detail for the procedures that cause the major impacts on animals and the related refinement control measures.

E10.10 End points should be specified in the licence and not determined by the NACWO or NVS, although indicating when their advice will be taken is helpful – see examples below.

E10.11 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 major adverse effects.

E10.12 Regarding surgery, consider both the adverse effects from conducting surgery under general anaesthesia and any longer-term consequences resulting from that surgery, e.g. neuropathic pain, consequent pathological changes.

E10.13 **Examples:**

E10.14 **Surgery (General adverse effects)**

- *Surgical procedures will be carried out aseptically. In the uncommon event of post-operative complications, animals will be killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than minor interventions. In the case of wound dehiscence, uninfected and minimally inflamed wounds may be re-closed on one occasion within 48 hours of the initial surgery.*
- *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 killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers;* ***and***
- *Any animal not fully recovered from the surgical procedure within 24 hrs (eating, drinking and return to normal behaviour) will be humanely killed.*

E10.15 **Example of the adverse effects associated with the model created by the surgical procedure**

- *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, the animal shows signs of kidney failure (hunched posture, starey coat, abnormal drinking and eating) it will be humanely killed.*

E10.16 **Example of general refinement control measures for protocols classified as mild**

- *Unless otherwise specified, the 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. Other procedures are expected to cause no more than transient distress and no lasting harm.*
- *If any of these procedures result in or induce evidence of suffering in an animal that is greater than mild and transient or in any way compromises normal behaviour the animal 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 [such as providing wet mash, additional warmth or topical treatments]. Enhanced monitoring and care will be instituted as advised by a veterinary surgeon or NACWO until the animal fully recovers. If there is not a rapid improvement within the working day, the animal will be humanely killed.*

E10.17 **Examples of refinement control measures for generation and breeding of genetically altered mice** can be found in the 'standard breeding protocols for genetically altered mice' under 'Application forms and guidance notes' https://www.gov.uk/guidance/research-and-testing-using-animals

E10.18 **Example of step by step adverse effects for skin carcinogenesis:**

***General Expected adverse effects and incidence***

- *The majority (>95%) of the animals are not expected to show signs of adverse effects that impact significantly on their general well-being. No more than 5% of animals are expected to show moderate clinical signs as a result of large epidermal tumours, when higher doses of anti-cancer drugs are used, or when a novel anti-cancer agent is administered. Very rarely the severity of these signs may be such that the humane end points may be reached.*
- *Unless otherwise specified, the 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.*
- *Mice will be killed if they show signs of ill health, such as piloerection and hunched posture, inactivity or inappetance for a period of 24hours. In addition, any animal that loses 15% of its body weight when compared to age matched controls or develops more serious clinical signs such as diarrhoea or dyspnoea will also be killed.*

***Surgical procedures:***

- *Surgical procedures will be carried out aseptically. In the uncommon event of post-operative complications, animals will be killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than minor interventions. In the case of wound dehiscence, uninfected and minimally inflamed wounds may be re-closed on one occasion within 48 hours of the initial surgery.*
- *Peri and post-operative analgesia will be provided; agents will be administered as agreed in advance with the NVS.*
- *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 killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers;* ***and***
- *Any animal not fully recovered from the surgical procedure within 24 hrs (eating, drinking and return to normal behaviour) will be humanely killed.*

***Skin biopsies:***

- *Infection is not expected because biopsies will be done aseptically.*

***Transgene inducing reagents:***

- *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.*
- *Animals will be closely monitored and killed if they show signs of ill health as described in 'General humane end-points and limits of severity' above.*

***Anticancer drugs:***

*The dose and duration of anticancer drug administration should result in only subtle non-specific signs indicative of loss of condition, mild reduction in activity and weight loss.*
*Animals will be closely monitored and killed if they show signs of ill health as described in 'General humane end-points and limits of severity' above.*

***Tumour growth:***

- *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.*
- *Tumours will be measured at appropriate intervals and animals will be monitored by daily physical examination during the expected critical periods of tumour growth.*
- *If the product of the maximum length and maximum breadth of tumours exceeds 15mm mean diameter 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 mice will be killed.*
- *Humane endpoints relating to the growth of internal tumours are judged by external signs, such as abdominal distension causing more than 10% increase in body weight when compared with age matched controls, a 20% loss in normal body weight when compared with age matched controls or a body condition score of below 3 (Mollie H. Ullman-Culleré and Charmaine J. Foltz, Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice Laboratory Animal Science Vol 49, No 3 June 1999), or other clinical signs, such as dyspnoea, jaundice, neurological signs, digestive disturbance.*

***Topical application:***

- *Repeated application of various agents in ethanol or acetone may occasionally cause excoriation of the skin.*
- *If excoriation of the skin is seen, skin painting will stop until the skin is completely healed.*
- *If the healing process is incomplete or excessive scar tissue develops the animal will be killed.*

E10.19 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 inform you of a breach of severity under standard condition 13 of their personal licence which you will subsequently need to notify to the Home Office under standard condition 18 of your project licence.

E10.20 **Re-use:**

E10.21 Read the Advice Note on 'Use, keeping alive and re-use'.
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/470008/Use

__Keeping_Alive_and_Re-use_Advice_Note.pdf

E10.22 When animals are transferred to a project licence for maintenance pending further use, you must specify, in the adverse effects section of the relevant protocol:

- likely adverse effects, controls and humane end-points for any 'maintenance pending further use' step of the protocol or the maintenance protocol as a whole. It is expected that the animals will remain in a suitable state of health and well-being, taking account of any model preparation the animal has previously undergone;
- controls on re-use, for example:
    - maximum number of times; and/or
    - perfomance standards e.g. patency of a cannula; and/or
    - 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.

E10.23 It is likely that most project licences authorising re-use will specify a combination of these factors.

## G: NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which will be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published. Examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals. In addition an example of a NTS prepared by the National Contact Points of EU Member States can also be found here: http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Recommendations%20for%20NTS.pdf

G1.1 **A good NTS will...**

- **Use clear, non-technical language** ('animals may experience low blood sugar levels' rather than 'animals may become hypoglycaemic')
- Clearly explain the benefits of the work to science, medicine, animals, the environment, education etc.
- Have a clear and concise explanation of the harms that will be experienced by the animal
    - Interventions described will include:
        - Injections
        - Dosing (e.g. oral, in water)
        - Blood or other sampling
        - Induction of experimental infections and illness
        - Anaesthesia/ repeated anaesthesia for non-invasive procedures (e.g. imaging)
        - Minor surgical procedures (that do not open a body cavity and have a minor impact on the animal) [should be defined/explained]
        - Major surgical procedures (may open abdomen, thorax, brain etc. or significantly interfere with a limb) [should be defined/explained]
    - Impacts on the animal/typical animal experience – for example
        - Minor impact on animal (rapid return to normal behaviour with minimal interventions)
        - Weight loss
        - Pain
        - Impaired senses
        - Impaired movement
        - Changes to appetite/drinking and/or vomiting/diarrhoea
        - Inability to feed, groom, nest or other fundamental behaviours
        - Single housing or other social harms such as depleted environmental enrichment
        - Whether animals may die as a result of the procedures and the nature of that death
    - Duration and frequency of harms
    - Age of animal
    - Mitigations
        - Monitoring regimes

    - Analgesia (“pain relief will be given as needed according to a regime recommended by the vet”)
    - Housing, enrichment, husbandry practices
- Provide a description of *how* the 3Rs are being addressed, not just that they *are* being addressed
- Be understandable to someone who has not read the main application

G1.2 **A good NTS will not...**

- Contain any information of a confidential nature or any information the publication of which may lead to the infringement of any person’s intellectual property rights
- Contain names or addresses or any other information from which the identity of the applicant or any other person can be ascertained
- Contain information (procedures, refinements, benefits etc) not provided elsewhere in the application
- Be very long
- Be cut and pasted from the main application without further editing
- Describe the effects on the animals as only “mild”, “moderate”,” severe” etc, without explaining what clinical signs/behaviours the animals will experience.

**(WORD LIMIT: 1000 WORDS)**

G1.3 Simple programmes of work with only mild or unclassified procedures are likely to require less detail than complex or severe work. Work involving cat, dogs, equidae, non-human primates, endangered or feral animals, or procedures likely to result in societal concerns, such as the growth of human gametes in animals should include more detail.

**Please complete the following:**

| **Project Title, Purpose & Duration** | **G2 will automatically populate from other sections of the form completed on ASPeL** |
|---|---|
| Key Words (max. 5 words) | G3 |
| Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | G4 |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | G5<br>• Describe clearly the benefits that will result from *this work.*<br>• You can briefly mention longer term benefits that *may* result – and clearly state the likelihood that these are realised.<br>• Do not make claims about the benefits that may arise from the work that are not genuinely attributable to the project itself (e.g. the project will result in new drugs to treat diabetes if the programme of work is a fundamental research programme looking at metabolic pathways). |

| | |
|---|---|
| What types and approximate numbers of animals do you expect to use and over what period of time? | G6<br>• This must be consistent with the licence.<br>• Explain the scientific rationale for requiring the number and type of animals. Specify the species to be used unless this would be very long (e.g. numerous freshwater fish species). Avoid very broad descriptors such as 'Avian'. |

| | |
|---|---|
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | G7<br>• Describe, in general terms, what animals will undergo:<br>  • explain how the animals are kept;<br>  • describe the procedures that they undergo. For example, if animals are dosed, which route(s)? And what does this involve? If animals undergo surgery, what does this involve?<br>*For example: During our 2-4 hour surgeries, we will typically make very small windows in the skull to gain access to the brain, implant tiny screws and probes no more than 4 mm long, then seal everything with dental cement.*<br>• Ensure that you describe the adverse effects in terms of what the animal is likely to experience<br>• Make sure you don't miss out harms. Include 'standard' techniques such as injections, imaging, general anaesthesia etc. All the harms you will inflict on the animals should be listed here. Include whether it is expected that animals may die as a result of the procedures.<br>• Don't just use unqualified general terms such as 'moderate' – make sure a lay reader will understand the degree and duration of harms and be clear what the highest level of severity expected and the proportion of animals likely to experience this level of suffering.<br>*For example: Mice will have minor surgery to implant a device under the skin that can release a medicine slowly. They are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital.*<br><br>**What will happen to the animals at the end?**<br>For example<br>• ***Methods of killing***<br>*For example: Animals will be killed by a humane method and tissues taken for analysis after death.*<br><br>• ***Keeping alive, setting free and/or re-homing***<br>*For example: Animals that are fully recovered at the end of procedures may be kept alive at the establishment (with the agreement of a vet), with a view to their re-use on procedures if appropriate and licensed. Some animals may be re-homed if it is in the best interests of the animal and they have been through the establishment's re-homing scheme. Otherwise animals will be killed humanely using an approved method.* |

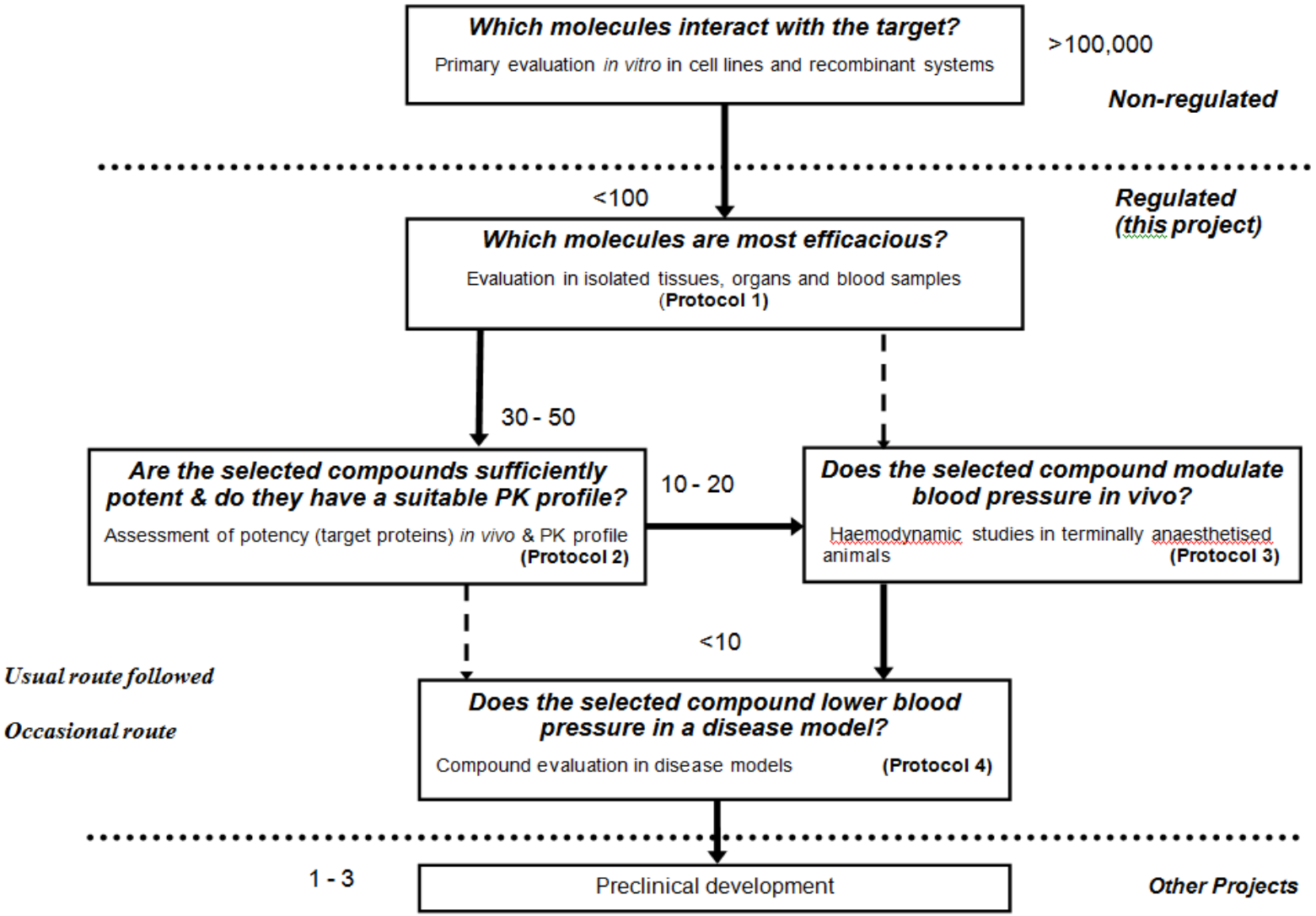
| **Application of the 3Rs** | |
|---|---|
| **Replacement**<br>State why you need to use animals and why you cannot use non-protected animal alternatives | G8<br>• Indicate the resources you have used to try to identify suitable alternative models.<br>• Explain what alternatives have been discounted and why.<br>• This should include referring to any relevant research that claims that non-animal alternatives are appropriate and why you can't achieve your objectives using such methods. |
| **Reduction**<br>Explain how you will ensure the use of minimum numbers of animals | G9<br>• Indicate in simple language the statistical principles used to design studies, where applicable.<br>• If you plan to re-use animals:<br>  • indicate the types of procedure on which an animal might be re-used;<br>  • explain the principles you will use to ensure animals are suitable, in both welfare and scientific terms, to be re-used;<br>  • indicate typically how many times an animal might be re-used. |
| **Refinement**<br>Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | G10<br>• The use of a particular species by other researchers is not an acceptable scientific justification.<br>• Give a scientific reason for using particular animal models. This needs to be a robust justification for models causing high degrees or durations of suffering.<br>• What are you going to do to refine the procedures, for example increased monitoring, supportive care, routine use of analgesia? |

## Appendices

Appendix 1 – Decision tree example A
Appendix 2 – Decision tree example B
Appendix 3 – Decision tree example C
Appendix 4 – HO minimum standards for aseptic surgery
Appendix 5 – Work with wild animals check list
Appendix 6 – Draft Advice Note on training requirements – To be published in the New Year

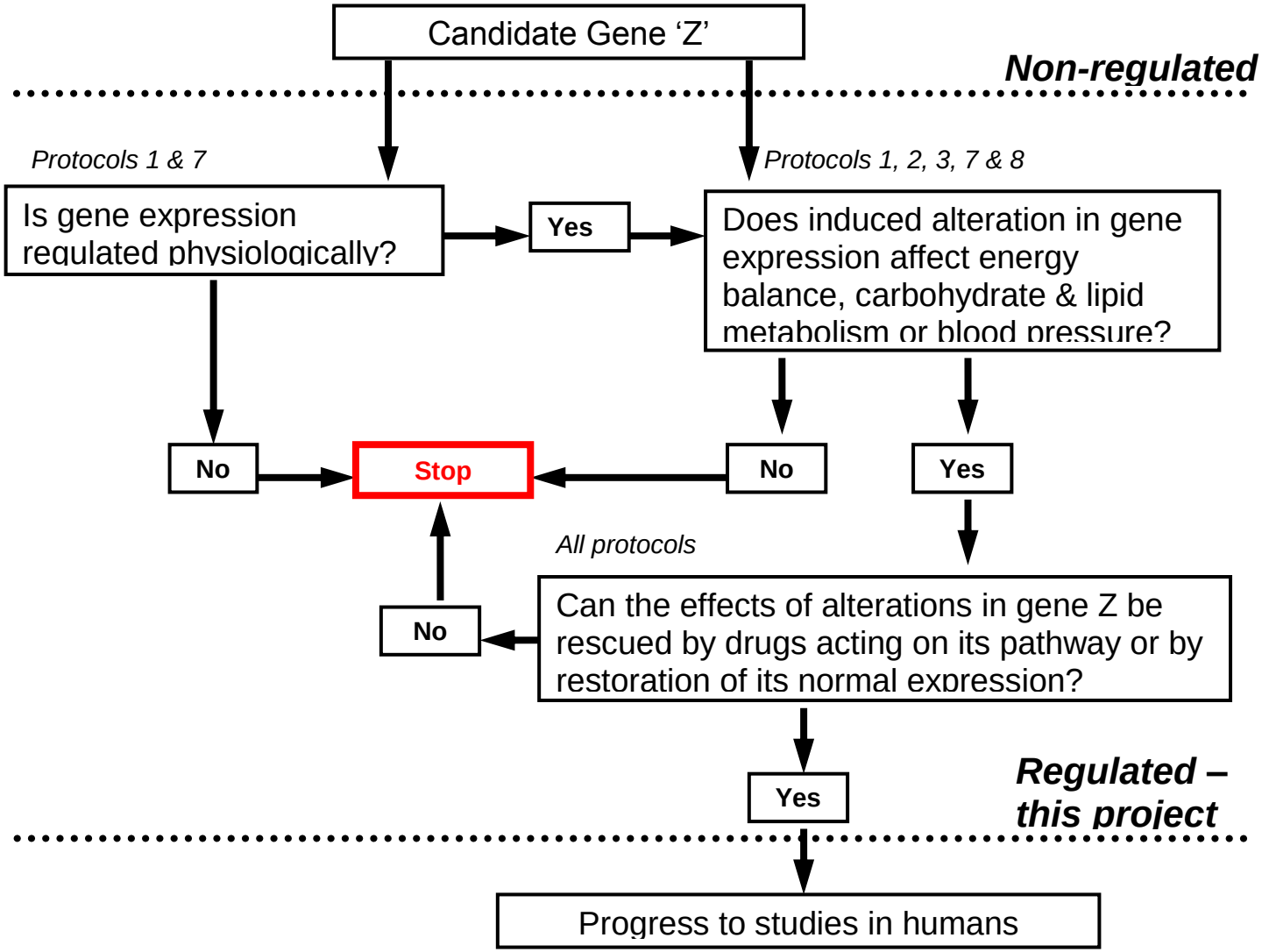
Appendix 1

**Decision tree example A - Drug development – novel antihypertensive agents**



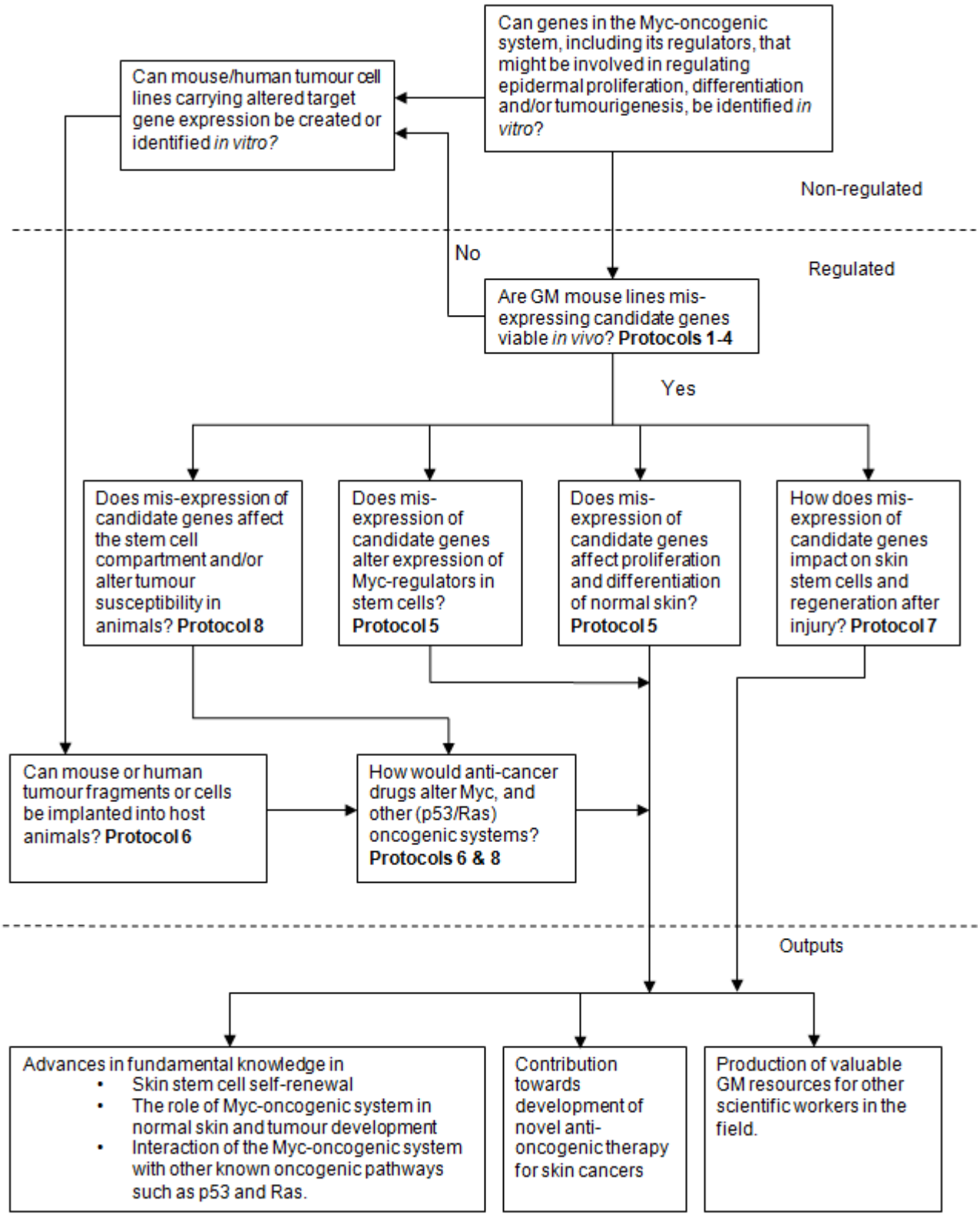
Appendix 2

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



## Appendix 3

### Decision tree example C - Epidermal stem cell self-renewal and differentiation



## Appendix 4

**Minimum Standards for Aseptic Surgery**

1. **Instruments**
    a. Instruments must be sterile (autoclaved) before starting aseptic surgery
    b. Use a fresh set for each operation
    c. If a fresh set is not available for batch surgery then a method of sterilizing instruments between each procedure must be in place
2. **Consumables:**
    a. All consumables (e.g. swabs, needles, suture materials) must be sterile and should be used within their guaranteed dates.
3. **Animal preparation**
    a. Clip and prep the animal for surgery in a separate place from the operating field.
    b. Clip and disinfect the incision site adequately using organic iodine or chlorhexidine solutions. Avoid alcohol as a disinfectant.
    c. Always cover the animal and bench with sterile drapes
4. **Surgeon preparation:**
    a. Use a clean gown (preferably sterile) or cover-all.
    b. Consider the use of protective clean head coverings
    **c.** Wash your hands and use sterile gloves – ensuring that they remain sterile as you put them on.
5. **During Surgery**
    a. Do not touch anything that is not sterile with sterile surgical gloves
    b. Only put down instruments on the sterile drape
    c. If you need to touch a non-sterile surface, eg anaesthetic machine, either change gloves immediately afterwards or use a sterile swab and immediately discard it

| Do's and Don'ts of Aseptic Technique | | |
|---|---|---|
| |  |  |
| **Plan** | • **Have all your surgical equipment ready in advance**<br>• **If in doubt consult the NVS or NACWO before starting any surgery** | • **Do not arrive unprepared and expect everything to be available and ready** |
| **Instruments** | • **Use sterilized surgical instruments and consumables for each surgery** | • **Alcohol/chlorhexidine does NOT sterilize the instruments.**<br>• **Do not put sterile instrument down onto the bench** |
| **Animal Preparation** | • **Prepare the skin of the animal adequately**<br>• **Use sterile drapes** | • **Alcohol is ineffective for skin preparation**<br>• **Don't contaminate the sterile field with non-sterile items** |
| **Surgeon** | • **Wear a clean gown or coverall**<br>• **Wash your hands**<br>• **Wear sterile gloves and change them if they become contaminated or punctured.** | • **DO NOT touch non-sterile surfaces after gloving up This includes the fur of the animal, anaesthetic machine, consumables and your phone!**<br>• **Do not allow the suture material to drag over a non-sterile surface** |