Home Office

Standard

Genetically Altered Rodent Protocols

June 2022

Version 2

Contents

Standard GA Rodent Protocols

Note: these General constraints are added automatically to all licences.

General constraints

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

Anaesthesia

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

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

General anaesthesia

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

Surgery

If authorised in this licence and unless otherwise specified:

- Surgical procedures should be carried out aseptically, to at least the published Home Office minimum
- In the uncommon event of post-operative complications, animals will be humanely killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than minor interventions. Minimally inflamed wounds without obvious infection may be re-closed on one occasion within 48 hours of the initial surgery. In the event of recurrence, NVS advice will be followed
- Peri and post-operative analgesia will be provided; agents will be administered as agreed in advance with the NVS
- All animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Uncommonly animals that fail to do so or exhibit signs of pain, distress or of significant ill health will be humanely killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers

- Any animal not fully recovered from the surgical procedure within 24 hrs (eating, drinking and return to normal behaviour) should be humanely killed

Administration of substances and withdrawal of fluids

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

Protocols

Protocol 1: Superovulation

Title

Superovulation

Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

Superovulation to generate ova, blastocysts, or embryos for re-derivation*/IVF*/cryopreservation. *

** Delete as appropriate*

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

Mild

What proportion of animals will experience this severity?

All animals.

Why are you proposing this severity category?

All animals will be administered substances to cause superovulation using standard routes which will cause mild, transient distress and no lasting harm.

Locations where this protocol can be carried out

Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?

Select all that apply.

Select objective(s)

Animals used in this protocol

Mice

Which life stages will be used during this protocol?

Select all that apply

- Adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes

How did these animals start their use?

Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Genetically altered animals (with or without associated wild types) for use in this protocol may be obtained from:

- This licence: Protocol X 'Breeding and maintenance of genetically altered animals' (mild); *
- This licence: [Protocol X 'Breeding and maintenance of genetically altered animals' (moderate)] [delete as appropriate]; * or
- other project licences with authority to breed and maintain genetically altered animals of a type authorised in this project, and to provide them for use on other projects. *

* *Delete as appropriate*

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

Yes

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

For example: Wild-type animals that have been genotyped using a regulated method and kept alive under the care of the NVS.

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

X (*Note this is maximum and not estimated.)*

What is the maximum number of uses of this protocol per animal?

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

1

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

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

GA mouse lines e.g., XXX for XXX.

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

No

Steps

Step 1 (mandatory)

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

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

Administration of agents to induce superovulation e.g., gonadotrophin, hormones or other agents administered typically twice but rarely up to four times by the following routes:

- intra-peritoneal (AA)
- subcutaneous (AA)

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 2 (mandatory)

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

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

Killing by a schedule 1 method.

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Fate of animals

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

Select all that apply

- Killed

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

No

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

The mice will typically experience two injections, approximately 48 hours apart. If embryos are needed these animals will also be mated. Mice will be killed by a Schedule 1 method at the appropriate time to harvest oocytes/blastocysts/embryos post-mortem.

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

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

Genetically altered animals used in this protocol are not expected to exhibit any harmful phenotype but if they do, they will be immediately killed by a Schedule 1 method.

Where the immune status of the animals might compromise health, they will be housed in a barrier environment.

Females will be of an appropriate size if they are to be mated. Over vigorous males will be replaced.

Any animal showing any deviation from normal health or wellbeing will be immediately killed by a Schedule 1 method.

Experimental design

What outputs are expected to arise from this protocol?

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

Oocytes, blastocysts, or embryos harvested post-mortem.

Will this protocol generate quantitative data?

No

Protocol justification

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

a) the most appropriate scientific approach?

Injection of hormones, such as gonadotrophin, is required to stimulate a greater number of ova to develop in the mouse ovary, which will generate higher numbers of oocytes/blastocysts/embryos (if mated). This enables a larger number of oocytes/blastocysts/embryos to be collected from a smaller number of females, which is a reduction.

---

b) the most refined for the purpose?

The doses and routes used are well validated and are not anticipated to produce any adverse effects, beyond transient discomfort due to the injections.

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

No clinical signs expected.

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

No clinical signs are expected.

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

N/A

Will you be administering substances for experimental purposes?

Yes

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

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

Substances used are commercially available for pharmaceutical use and are known not to have toxic side effects.

How will you determine an appropriate dosing regimen?

Include routes, dosage volumes, frequencies, and durations.

Routes, dosage volumes, frequencies and durations will be obtained from published literature. No more than 20ml/kg will be administered by either route.

Protocol 2: Embryo recipients

Title

Embryo recipients

Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

To implant and grow embryos and blastocysts in recipients of an appropriate health status.

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

Moderate

What proportion of animals will experience this severity?

…% of animals will experience moderate severity.

Why are you proposing this severity category?

Animals undergoing surgical embryo transfer will experience short-lived post-operative pain and discomfort.

Animals undergoing non-surgical embryo transfer will experience no more than mild transient discomfort and no lasting harm.

Locations where this protocol can be carried out

Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?

Select all that apply.

Select objective(s)

Animals used in this protocol

Mice

Which life stages will be used during this protocol?

Select all that apply

- Adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

No

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

Yes

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

For example: We may re-use wild-type mice that have been genotyped by a regulated method. We may re-use mice that have been implanted previously by a non-surgical method.

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

X (*Note this is maximum and not estimated.)*

What is the maximum number of uses of this protocol per animal?

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

1 (Note: If re-using mice previously implanted non-surgically, this number will be greater than 1)

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

No

Steps

Step 1 (mandatory)

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

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

Embryos and blastocysts will be implanted surgically (AB) or non-surgically (AA/AB) into the reproductive tract of a mouse rendered pseudo-pregnant by mating with a sterile male.

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 2 (mandatory)

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

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

All surgically implanted mice will be killed by a Schedule 1 method.

Non-surgically implanted mice will be killed by a Schedule 1 method or kept alive for potential re-use.

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Fate of animals

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

Select all that apply

- Killed

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

No

- Kept alive

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

Female mice will be rendered pseudo-pregnant by mating with a sterile male. Implantation will either be non-surgical, with or without brief anaesthesia, or by a surgical procedure under general anaesthesia. If embryos are required the dam will be humanely killed by a schedule 1 method, otherwise they will be allowed to give birth naturally and raise the litter.

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

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

See General constraints.

Females will be of an appropriate size if they are to be mated.

Over vigorous males will be replaced.

Experimental design

What outputs are expected to arise from this protocol?
For example, test results, phenotypic information, or products.

Genetically altered mice*/embryos*

* *Delete as appropriate*

Will this protocol generate quantitative data?

No

Protocol justification

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

a) the most appropriate scientific approach?

The only method currently available for allowing GA embryos to develop is to implant them into the uterus of a pseudo-pregnant mouse.

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b) the most refined for the purpose?

Non-surgical embryo transfer methods will be used where the success rate matches that of surgical embryo transfer methods or is satisfactory.

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

Animals are expected to make an uneventful recovery from any surgery. No other clinical signs are expected.

Why scientifically do the animals need to suffer to this degree?
This protocol follows current standard procedures.

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

N/A

Will you be administering substances for experimental purposes?

No

Protocol 3: Vasectomy

Title

Vasectomy

Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

To produce sterile male mice for mating to obtain pseudo-pregnant female mice to be used for embryo transfer.

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

Moderate

What proportion of animals will experience this severity?

All animals.

Why are you proposing this severity category?

All animals will undergo surgery for vasectomy and will experience short-lived post-operative pain and discomfort.

Locations where this protocol can be carried out

Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?

Select all that apply.

Select objective(s)

Animals used in this protocol

Mice

Which life stages will be used during this protocol?

Select all that apply

- Adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

No

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

Yes

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

For example: Wild-type mice that have been genotyped by a regulated method.

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

x (*Note this is maximum and not estimated.)*

What is the maximum number of uses of this protocol per animal?

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

1

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

No

Steps

Step 1 (mandatory)

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

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

Vasectomy (AB)

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 2 (optional)

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

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

Killing using a Schedule 1 method.

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Fate of animals

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

Select all that apply

- Killed

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

No

- Kept alive

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

After a post-op recovery period (minimum 2 weeks) sterility will be assessed, e.g., by mating the vasectomised male with normal females. Typically, mice are then kept alive under the direct supervision of a veterinary surgeon for mating with embryo recipients to render them pseudo-pregnant until they are too large or too old, when they will be humanely killed.

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

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

See General constraints.

Experimental design

What outputs are expected to arise from this protocol?

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

Sterile male mice.

Will this protocol generate quantitative data?

No

Protocol justification

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

a) the most appropriate scientific approach?

Sterile male mice are required to induce pseudopregnancy in females prior to embryo transfer. Surgical vasectomy is a reliable method to produce sterile males.

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b) the most refined for the purpose?

Where possible genetically altered sterile males will be used instead of vasectomised males.

Animals will not be used for mating until they are regaining weight and they are showing no adverse signs following surgery.

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

No clinical signs are expected, apart from non-eventful recovery from surgery.

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

Where possible genetically altered sterile males will be used instead of vasectomised males. When this is not possible, surgical vasectomy using a scrotal approach is currently the most refined standard procedure.

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

N/A

Will you be administering substances for experimental purposes?

No

Protocol 4: Breeding and maintenance of GA mice (mild)

Title

Breeding and maintenance of genetically altered mice (mild)

Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

To produce, maintain and provide genetically altered mice.

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

Mild

What proportion of animals will experience this severity?

Choose one of the following:

All animals

OR

Few – we expect the phenotype(s) to be sub-threshold and genotyping will generally be undertaken using surplus material from ear notching for identification.

Why are you proposing this severity category?

Animals are not expected to show harmful phenotypes that are more than minor or transient.

Locations where this protocol can be carried out

Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?

Select all that apply.

Select objective(s)

Animals used in this protocol

Mice

Which life stages will be used during this protocol?

Select all that apply

- Embryo and egg
- Neonate
- Juvenile
- Adult
- Pregnant adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes

How did these animals start their use?

Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Genetically altered animals for use in this protocol may be obtained from other projects with authority to breed and maintain genetically altered animals of a type authorised in this project, and to provide them for use on other projects.

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

No

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

X (*Note this is maximum and not estimated.)*

What is the maximum number of uses of this protocol per animal?

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

1

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

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

Genetically altered mice XXX for XXX.

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

No

Steps

Step 1 (mandatory)

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

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

The production and birth of genetically altered offspring arising from:

- breeding by conventional breeding methods
- generation of genetically altered embryos and blastocysts by in vitro manipulation and/or fertilisation with implantation into an embryo recipient and intrauterine development*

** Delete if not part of the project*

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 2 (optional)

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

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

Tissue biopsy to determine genetic status by one of the following methods:

- ear biopsy (AA/ABL)
- blood sampling (AA/ABL)
- hair sampling (AA/AB)
- mouth swabbing (AA)

Rarely, due to technical problems during analysis, a second sample may be taken using the least invasive method.

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 3 (mandatory)

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

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed

under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Maintenance to the age of 15 months.

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 4 (optional)

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

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

Terminal procedures:

- Exsanguination (AC) with killing by a Schedule 1 method *
- Perfusion fixation (AC) *
- Schedule 1 killing

* *Delete as applicable*

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Fate of animals

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

Select all that apply

- Killed

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

No

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

Please state the relevant protocol.

For example:

Offspring will be used on experimental protocols A and B.

Adult mice may be used for superovulation under protocol 1.

- Continued use on other projects

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

Animals may be used for natural mating on a number of occasions.

Animals produced under this protocol are not expected to exhibit any harmful phenotype.

Offspring will be maintained by methods appropriate to their genetic alteration until they reach a maximum of 15 months of age.

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

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

Some animals may have an altered immune system making them more susceptible to infection. Animals with altered immune status will be housed in a barrier environment thereby minimising the likelihood of compromising health.

Any animal will be immediately killed by Schedule 1 method if it shows signs of suffering that is greater than minor and transient or in any way compromises normal behaviour unless moved on to another protocol for a specific purpose (continued use).

Animals exhibiting any unexpected harmful phenotypes will be killed (Schedule 1), or in the case of individual animals of particular scientific interest, advice will be sought promptly from a Home Office inspector.

Other than those described in the strain-specific adverse effects above, animals are not expected to die because of any authorised genetic alteration. A small number of animals, living beyond the neonatal period (5 days – before which ASRU does not require you to report any mortality), may suddenly and unexpectedly die having shown no preceding clinical signs indicative of impending death. Unless otherwise indicated, such deaths, should they occur, are unlikely to be related to the genotype. However, as per the published ASRU Advice Note on Severity Assessment of GA animals, should the mortality rate (age-matched) of the genetically altered strain rise beyond that present in the background source breeding colony, this will be reported under PPL standard condition 18.

Experimental design

What outputs are expected to arise from this protocol?

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

Genetically altered mice.

(Note: If you are using this protocol to produce tissues for in vitro use you should include mention of the expected outputs from that use and answer Yes to the question below).

Will this protocol generate quantitative data?

No

(Note: If you are using this protocol to produce tissues for in vitro use that will generate quantitative scientific data you should answer 'Yes' to this question and complete the boxes which appear).

Protocol justification

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

a) the most appropriate scientific approach?

Genetically altered mice of these types are required to achieve the scientific aim of this project.

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b) the most refined for the purpose?

The act of natural mating is not regulated per se and no animals on this protocol are expected to show clinical signs that could exceed mild.

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

No significant clinical signs are expected.

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

No significant clinical signs are expected.

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

N/A

Will you be administering substances for experimental purposes?

No

Protocol 5: Breeding and maintenance of genetically altered mice (moderate)

Title

Breeding and maintenance of genetically altered mice (moderate)

Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

To produce, maintain and provide genetically altered mice.

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

Moderate

What proportion of animals will experience this severity?

Approximately X% of animals are likely to experience moderate levels of severity.

Why are you proposing this severity category?

For example:

We will be using strain X of genetically altered mice and this strain will show progressive ...{...}. from {...} weeks of age until reaching {....} at 15 weeks of age.

Repeat this for all the strains that are likely to develop a phenotype.

Locations where this protocol can be carried out

Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?

Select all that apply.

Select objective(s)

Animals used in this protocol

Mice

Which life stages will be used during this protocol?

Select all that apply

- Embryo and egg
- Neonate
- Juvenile
- Adult
- Pregnant adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes

How did these animals start their use?

Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Genetically altered animals (with or without associated wild types) for use in this protocol may be obtained from

- This licence: Protocol X (Breeding and maintenance of genetically altered mice (Mild); or
- other projects with authority to breed and maintain genetically altered animals of a type authorised in this project, and to provide them for use on other projects.

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

No

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

X (*Note this is maximum and not estimated.)*

What is the maximum number of uses of this protocol per animal?

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

1

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

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

For example: We will be using genetically altered mice strain X that overexpresses/does not expresses gene X.

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

Yes

Why are each of these harmful phenotypes necessary?

For example: To determine whether gene X plays a role in the development of disease X, we will create mice that either don't express the gene or overexpress the gene which leads to the clinical findings {...}.

How will you minimise the harms associated with these phenotypes?

Ensure that you include any humane endpoints that you will use.

For example: Some lines may be embryonic lethal or lethal before adulthood (e.g., XXX), and such lines will be made as conditional knockouts or be maintained as heterozygotes.

Steps

Step 1 (mandatory)

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

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

The production and birth of genetically altered offspring arising from:

- breeding by conventional breeding methods
- generation of genetically altered embryos and blastocysts by in vitro manipulation and/or fertilisation with implantation into an embryo recipient and intrauterine development*

** Delete if not part of the project*

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 2 (optional)

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

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

Tissue biopsy to determine genetic status by one of the following methods:

- ear biopsy (AA/ABL)
- blood sampling (AA/ABL)
- hair sampling (AA/AB)
- mouth swabbing (AA)

Rarely, due to technical problems during analysis, a second sample may be taken using the least invasive method.

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Step 3 (Mandatory)

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

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

Maintenance to the age of 15 months.

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

Yes

What are the likely adverse effects of this step?

State the expected adverse effect, including the likely incidence, and the anticipated degree and duration of suffering.

Add details for each strain or type of GAA. For example:

Strain X will show the following clinical signs: overtly normal up to 4 weeks of age;

- progressive {....} until reaching. {....} .. at 15 weeks of age.

How will you monitor for, control, and limit any of these adverse effects?

If adverse effects can't be prevented, how will you attempt to ameliorate their initial signs?

Add details for each strain or type of GAA. For example:

Strain X: Breeding stock will be only allowed one breeding cycle and offspring will be killed or transferred to an experimental protocol before the age of {x weeks}. During that time, they should show no more than XXX.

What are the humane endpoints for this step?

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

Add details for each strain or type of GAA. For example:

Strain X: Breeding stock will be killed after the first litter is weaned, and before onset of {...}. Offspring will be killed before 4 weeks of age or at the onset of clinical signs if earlier unless required for experimental use when they will be transferred for continued use.

Step 4 (optional)

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

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

Terminal procedures:

- Exsanguination (AC) with killing by a Schedule 1 method *
- Perfusion fixation (AC) *
- Schedule 1 killing

** Delete as applicable*

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

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

No

Fate of animals

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

Select all that apply

- Killed

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

No

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

Please state the relevant protocol.

For example:

Offspring will be used on experimental protocols A and B.

Adult mice may be used for superovulation under protocol 1, provided that they are not showing any adverse clinical signs.

- Continued use on other projects

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

Animals will be bred by natural mating.

Maintenance of animals by methods appropriate to their genetic alteration until they reach a maximum of 15 months of age.

For example: Strain X: mice will show XXX and will be humanely killed before the age of XXX or be transferred to another protocol for continued use.

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

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

Any mouse that shows XXX will be immediately killed (Schedule 1)

Some animals may have an altered immune system making them more susceptible to infection. Animals with altered immune status will be housed in a barrier environment thereby minimising the likelihood of compromising health.

Any animal will be immediately killed by Schedule 1 method if it shows signs of suffering that is likely to exceed those detailed for the genetic construct.

Animals exhibiting any unexpected harmful phenotypes (i.e., not normally associated with the GA construct) will be killed (Schedule 1), or in the case of individual animals of particular scientific interest, advice will be sought promptly from a Home Office Inspector.

For each line, a detailed phenotypic assessment will be made, for inclusion in a mouse passport.

Animals are not expected to die because of any authorised genetic alteration. A small number of animals, living beyond the neonatal period (5 days for mice and rats – before which ASRU does not require you to report any mortality), may suddenly and unexpectedly die having shown no preceding clinical signs indicative of impending death. Unless otherwise indicated, such deaths, should they occur, are unlikely to be related to the genotype. However, as per the published ASRU Advice Note on Severity Assessment of GA animals, should the mortality rate (age-matched) of the genetically altered strain rise beyond that present in the background source breeding colony, this will be reported under PPL standard condition 18.

Experimental design

What outputs are expected to arise from this protocol?

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

Genetically altered mice.

(Note: If you are using this protocol to produce tissues for in vitro use you should include mention of the expected outputs from that use and answer Yes to the question below).

Will this protocol generate quantitative data?

No

(Note: If you are using this protocol to produce tissues for in vitro use that will generate quantitative scientific data you should answer 'Yes' to this question and complete the boxes which appear).

Protocol justification

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

a) the most appropriate scientific approach?

Genetically altered mice are needed for XXX.

---

b) the most refined for the purpose?

For example: We need mice aged { } with genetic alterations XXX to achieve objective A.

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

For example: We need mice aged { } with genetic alterations XXX to achieve objective A.

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

For example: We need animals that are showing {clinical signs} in order to { }…

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

For example: An earlier humane end point cannot be achieved because XXX.

Will you be administering substances for experimental purposes?

No
