Home Office

NON-TECHNICAL SUMMARY

Breeding and maintenance of germ free and gnotobiotic animals service licence

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (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)

Key words

Breeding, Microbiome

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this project is to act as a service licence to breed, maintain and supply mice for microbiome research. Microbiome research investigates the impact of gut bacteria in human disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This work will ensure a steady supply of germ-free mice and mice with known germs for the use on other experimental licences. These mice will be used to understand the role of the gut bacteria (mouse microbiome) in disease and how it can be changed for therapeutic effect. This could include identifying mixtures of beneficial, health-associated bacteria which have the potential to be used to treat diseases such as cancer and digestive disorders in clinical trials.

What outputs do you think you will see at the end of this project?

The outputs from this project are:

a) production of demand matched animals for research thereby reducing animal wastage,

b) the production of animals with known gut bacteria required to answer specific research questions and

c) the communication of information arising from the breeding and maintenance of these animals to other researchers so that they might benefits from the knowledge.

Who or what will benefit from these outputs, and how?

In the short-term, mice produced on this project will enable microbiome experimental studies to be carried out by researchers on other projects.

In the long-term this research may lead to the development of microbiome therapies to benefit patients with diseases such as types of cancer including melanoma and lung cancer and intestinal disorders such as ulcerative colitis and crohn's diesease.

How will you look to maximise the outputs of this work?

Knowledge generated from the breeding and maintenance of these animals will be communicated with collaborators and other researchers working on similar projects.

Species and numbers of animals expected to be used

- Mice: 2500 germ-free and 1500 gnotobiotic

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are ideal for studying the microbiome. The mice we will breed and maintain are wild type (containing no genetic alterations) however they contain no microorganisms such as bacteria, fungi and viruses.

The project breeds of mice at all life stages, excluding aged mice, as mating and weaning are required to establish mouse colonies.

Mice share the same types of bacteria with humans and are small and easily housed in an extra clean environment in the animal house. The housing we use is called an isolator and everything that enters is sterile (e.g. the water, air, bedding etc.) to create a germ-free bubble.

Typically, what will be done to an animal used in your project?

To maintain a germ-free colony it is necessary to breed mice within germ-free bubbles called isolators.

Mice will be mated in pairs (one male and one female) or trios (two females and one male) and productivity monitored with germ-free mice typically mated up to an age limit of 12 weeks. Offspring are maintained to 5-8 weeks of age before being transferred to other projects that require the use of these animals.

In some cases, germ-free mice are given specific gut bacteria from other mammals, to produce gnotobiotic mice with a known gut bacteria. The gut bacteria is administered via the mouth into the stomach through a tube.

What are the expected impacts and/or adverse effects for the animals during your project?

Breeding of animals is not expected to produce any adverse effects. For natural breeding issues, such as littering problems, advice will be sought from a Named Animal Care and Welfare Officer or the Named Veterinary Surgeon.

Germ-free mice can develop abnormalities within the gut such as an enlarged caecum (pouch connecting the small and large intestine) as they age. In turn, the size of the caecum may trigger a torsion of the intestine (twisted caecum). This causes chronic diarrheal status and death of the mouse in up to 3% of the animals. The age of breeders, number of litters and breeding performance will be monitored to minimise the impact of the enlarged caecum with age.

Mice will be checked at least twice per day and those observed to be less active, showing poor coat condition, abdominal distension, or other clinical signs that in any way compromises normal behaviour will be humanely killed.

Very rarely, gastrointestinal tract damage may occur during administration of microbiota by the mouth. This allows toxic material from the live bacteria to be released into the bloodstream causing infection and possible death within approximately four days. Animals displaying signs of infection, such as acute piloerection and lack of responsiveness when provoked, will be humanely killed. To minimise damage caused, the bacteria will be administered under anaesthesia.

Gnotobiotic mice, containing a known microbiome, do not develop the same intestinal issues as germ-free mice.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mouse: Mild 97%

Moderate 3%

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

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The project aims to supply mice for use on other experimental projects utilising animals with altered gut microbiomes and therefore must use breeding of animals to achieve this.

Which non-animal alternatives did you consider for use in this project?

We use a range of human assays, within our laboratory, to complement this research and reduce the number of animals required. These assays use human cells from blood samples to measure the behaviour of gut bacteria.

Why were they not suitable?

Using human cells from blood samples for laboratory assays does not fully replicate the complexities of the gut barrier and the interactions which occur between gut bacteria and the immune system (white blood cells) within the gut.

Particularly in cancer research, it is impossible to measure the whole body effects of particular gut bacteria on the growing tumour within these assays.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The purpose of this service licence is to breed and supply mice for microbiome research performed on other project licences.

The number of animals are estimated based on our in-house knowledge of animals required for these projects and experience of breeding efficiency and litter sizes in germ-free mice.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

These animals are to be used on other project licences where the experimental design has utilised design tools such as the NC3R's Experimental Design Assistant and other computational analyses to use the minimum number of mice possible whilst maintaining the integrity of the results.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We are continually reviewing ways to make the breeding of our colonies more efficient in producing sufficient offspring whilst keeping the numbers of breeding pairs to a minimum.

The use of a range of human laboratory assays to complement this research will increase our understanding of the behaviour of particular bacterial species. By screening these bacteria in laboratory assays we are able to select only the best 'health associated' bacteria reducing the number of bacterial therapies to be tested in animal models.

This will subsequently help to reduce the number of animals required for this project.

Refinement

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

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project utilises breeding techniques to maintain germ-free and gnotobiotic mouse colonies. Breeding is not anticipated to cause any pain, suffering, distress or lasting harm to the animals.

In order to produce new mouse colonies a minority of germ-free mice will be given mammalian gut bacteria orally to produce gnotobiotic mice with a known gut flora (bacteria).

Why can’t you use animals that are less sentient?

Less sentient animals such as fish or frogs are not appropriate for microbiome research as these differ in gut bacterial species and are not as easily housed under germ-free conditions.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

During breeding of animals the age of breeders, number of litters and breeding performance will be closely monitored.

To reduce the likelihood of the enlarged caecum with age in germ-free mice breeding will be limited to 12 weeks of age for males and 18 weeks for females (if pregnant at 12 weeks).

Mice will have two daily welfare checks which include observing that animals are healthy and can move freely in every cage, have sufficient food and water and the isolator temperature/humidity readings are appropriate. These details are recorded on observation sheets within a day book for assessment of an individual animal's health status.

For the minority of mice which receive oral administration of a mammalian microbiome, this procedure has been refined to be carried out under anaesthetic to reduce the likelihood of any infection due to bacteria entering the bloodstream during the process. Post procedure checks are carried out.

Following in depth screening of the behaviour of bacterial species we will continue to work with animal care staff to assess the requirement for anaesthetic use.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure best practices we follow the updated ARRIVE guidelines [PLoS Biol 2020 18(7): e3000410]

We will follow the PREPARE guidelines to ensure all best practices are followed by researchers who use animals bred on this licence. [Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.]

In addition, we have consulted the Home Office guidelines published on efficient breeding of rodents and follow updates to best practice guidelines on The Laboratory Animal Science Association, LASA website.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will use the NC3Rs website to gather up to date information about advances in animal research. This information will be disseminated to anyone involved with this licence including the project licence holder (PPLH), personal licence holders working under the licence (PILs)and named animal care and welfare officers (NACWOs) and implemented where appropriate.