Home Office

NON-TECHNICAL SUMMARY

Breeding and maintenance of genetically altered zebrafish

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

embryos, genetically altered, cryopreservation, maintenance

Animal types	Life stages
Zebra fish (Danio rerio)	Embryo and egg, Neonate, Juvenile, Adult

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?

This licence will provide authority for users to create and maintain genetically altered zebrafish, including cryopreservation, the process of preserving biological materials by cooling them to low temperatures.

All protected animals generated under this licence will be used to maintain genetically altered lines, to produce the required embryos/larvae for pre-protected stage experimental work, and/or to allow for phenotyping through growth measurements.

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?

The use of genetically altered zebrafish provides a powerful and efficient model for investigating human diseases. This license will support multiple research groups across diverse areas of biological significance, with the shared goal of expanding knowledge and creating translational research, ultimately advancing therapeutic discoveries.

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

1. Academic knowledge specific to each user group

2. Publications and presentations to the wider scientific community

3. Potential to inform new therapeutic programmes

4. Translation into higher species, e.g. mice and rats

5. Generation of genetically altered zebrafish that may be made available to the wider scientific community

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

1. Research groups and collaborators within the Establishment

2. External research groups and collaborators, potentially worldwide

3. Wider scientific community through published papers, presentations and similar.

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

Users of this licence will be encouraged to share their findings, including negative results, through publications and presentations within their field of research as a minimum.

All transgenic lines created and maintained under this licence will be available to other researchers, provided appropriate justification is provided.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 105000

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.

Zebrafish are a well-established animal model, utilising a species thought to be less sentient than other animal models such as mice and rats. Their externally laid, transparent embryos allow researchers to track development, as well as facilitating easy insertion or deletion of genetic materials to create genetically altered lines.

At less than 5 days post-fertilisation, zebrafish embryos and larvae are not considered to be a protected species under the Animal (Scientific Procedures) Act as they are not capable of independent feeding at this point, and are presumed to have limited capacity for suffering. Most of the users' experimental work will occur during this life stage. Nonetheless, we expect all users to apply high standards of husbandry to ensure optimal welfare and support the generation of robust scientific data.

Adult zebrafish are maintained to enable propagation of the genetically altered lines.

In a small set of pre-determined cases, some zebrafish may be anaesthetised to facilitate accurate body measurements for growth monitoring at varying times throughout their life.

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

For the vast majority of zebrafish used under this licence, they will be bred either to produce embryos for experimental work or for the propagation of the genetically altered line. Some of these genetically altered lines will require genotyping, which is the process of determining the DNA of individual fish to determine which variants of specific genes they have. Where genotyping is necessary, it will be carried out in the most refined manner available.

A small set of zebrafish will be anaesthetised to allow for accurate body measurements to be taken to facilitate growth trends.

Tissue may be taken from humanely euthanised zebrafish for analysis.

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

Animals that undergo anaesthesia are expected to make a full recovery within 30 minutes of the anaesthesia being removed. Any that do not make a full recovery within 30 minutes will be humanely euthanised.

Where genotyping is carried out via tail fin tissue biopsy, fish will be monitored for any signs of infection or abnormal swimming behaviours. Any fish that deviates from normal health and/or behaviour will be humanely euthanised.

Fish undergoing genotyping via tail fin amputation will receive suitable analgesia, as advised by the Named Veterinary Surgeon.

Fish exhibiting any unexpected, harmful observable traits will be humanely killed, or in the case of individual fish of particular scientific interest, advice will be sought promptly from a Home Office Inspector.

During genotyping, fish will be isolated for a minimal amount of time. As fish live in shoals, the time in isolation must be as short as practicably possible. For circumstances where results take an unduly long amount of time, housing of these fish with easily distinguishable shoals will be utilised.

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)?

100% Mild

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

To create genetically altered zebrafish embryos and larvae, adult animals are required for breeding. Zebrafish embryos and larvae will be extensively used to investigate the interaction between individual cells and molecules in a non-protected vertebrate animal model. In some cases, growth measurements will be taken to track the development of genetically altered zebrafish lines.

While non-animal alternatives can be employed to investigate certain biological mechanisms, especially at a more simplified or molecular level, they do fall short in replicating the full complexity of

interactions present in a living organism. Zebrafish models are uniquely suited to reveal how genetic modifications influence normal development and disease processes within an intact, dynamic system.

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

Users of this service licence will be expected to remain informed about emerging technologies and methodologies that offer viable alternatives to zebrafish models, particularly in vitro systems that can replicate key biological processes. As part of a commitment to the principles of the 3Rs, researchers will actively evaluate and adopt approaches that minimise the use of animal models wherever scientifically appropriate, ensuring both ethical responsibility and scientific rigour.

Why were they not suitable?

Whilst useful in establishing simple interactions in vitro, our models are not yet suitably advanced to allow the full interactions to be modelled and predicted with certainty in an ex vivo setting.

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 projected number of animals required is informed by historical data from the previous service PPL, user feedback regarding anticipated usage under the licence, estimated larval requirements, and the number of adult zebrafish needed for breeding. This also includes individuals tracked for growth and developmental studies.

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

Given that the outputs from this service licence will not be numerical data per se, experimental design calculations have not been possible. However, the design of our breeding strategies will be dictated by user demand and will be rigorously reviewed during the lifetime of this licence to ensure genetically altered lines are not overproduced.

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

All breeding under this licence will be stringently managed in consultation with highly experienced technicians. Careful management of the fish lines will help to reduce the number of animals produced;

i.e. animals will only be produced where there is a justifiable need. Where possible, all larvae bred will be shared between research groups.

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.

Genetically altered zebrafish will either be created or maintained under this licence. Only genetic alterations that at most are predicted to cause mild impact on animal welfare will be bred under this licence.

Where a new genetic alteration is made, the resulting progeny will be monitored carefully during the non-protected phases to identify any mutations that could potentially impact animal welfare. These animals will be additionally carefully assessed for any welfare impacts once they reach free-feeding stages for any signs of suffering.

Any animals' welfare found to be impacted more than mildly will be euthanised humanely, and the breeding of those animals will be reassessed.

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

Experimentally, zebrafish embryos and larvae will be utilised. However, to produce these, adult zebrafish are required. In a small number of cases, the impact of the altered genetics on growth will be recorded via measurements taken under anaesthesia.

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

All zebrafish will be provided with environmental enrichment, where appropriate.

Where animals will be subject to painful procedures, for example, fin biopsy to obtain genetic material for genotyping, we will obtain Named Veterinary Surgeon advise on appropriate analgesia regimens to minimise any pain and suffering.

We will also continue to explore alternative methods, such as the use of the ZEG machine (which allows for the genotyping of embryos, prior to the point they become protected) and swabbing to obtain genetic material for analysis.

Anyone working with zebrafish will be trained in the careful handling, and the appropriate selection and use of equipment to minimise the risk of injury during handling and netting.

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

We will follow guidance published by notable bodies regarded as experts in the field, such as FELASA .

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

We will consult with leaders in the field of zebrafish welfare, husbandry and breeding, including but not restricted to FELASA and the NC3Rs, to implement any improvements in animal welfare and non-animal alternatives. Additionally, Establishment staff are encouraged to attend zebrafish-related seminars and workshops to enhance their knowledge.

The establishment maintains active membership in the Zebrafish Husbandry Association, a global community dedicated to sharing best practices and advancing scientific research involving zebrafish.