NON-TECHNICAL SUMMARY (NTS)

Project Title	Using developing zebrafish to understand disease and develop therapies
Key Words	zebrafish, development, disability, therapy, mechanism
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(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 aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

This project aims to increase our understanding of diseases, provide disease models for therapeutic discovery and development, and investigate the processes and molecules involved in vertebrate development. The project focuses on rare inherited diseases, which together affect about one in every 10 people, even though each disease has an incidence of less than 1 in 2000 people. They frequently cause premature death in childhood and the vast majority have no approved treatments. We will also study diseases that have a more complex origin, in which environmental factors contribute significantly. There is a huge need to find treatments

for both inherited and complex diseases. To help us find treatments, we model the disease in fish and investigate those fish to find out which aspects of the disease we should be targeting with treatment. As many of the diseases we study affect children, and because we need to understand the function of proteins in the whole animal, externally developing embryos are best suited for our studies.

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)?

We expect to increase our understanding of several diseases, predominantly neurodegenerative diseases that affect children, and move closer to a treatment. For example our drug discovery and testing studies, enabled by the large number of zebrafish embryos we can generate and the small quantities of compound needed for treating embryos and larvae, will speed up the selection of the most promising compounds for testing on mammalian models.

What types and approximate numbers of animals do you expect to use and over what period of time?

We use the zebrafish as our experimental model. The embryos develop externally from the mother and do not become free-feeding and regulated by the Home Office until 5 days post-fertilisation. The diseases we model predominantly affect embryos at unregulated stages so these studies are not regulated. However, we require a Project Licence as we use genetically-altered zebrafish (which themselves do not have the disease), and we need to generate and characterise some new disease models and we do not know at what age they will show the disease. At regulated ages, we expect to generate 8000 genetically altered zebrafish that are older than 5 days post-fertilisation over a 5 year period. We already know that these adult fish will not be harmed by their genetic-alteration. Up to 2500 would undergo mild procedures with good husbandry to reduce the number of animals used overall and to enable identification of genetically altered fish.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The maximum severity we expect is moderate. When we generate new disease models, we will prevent severe harm by limiting the number of fish and the maximum length of time it can suffer. Suffering will be limited and maximum information gained by culling followed by tissue analysis. We will also perform a mild procedure - removal of part of the fin for genotyping and extracting sperm and eggs for in vitro fertilisation. These protocols will be performed under anaesthetic. Analgesia will be given to fish from which fin tissue is taken. Hence, discomfort is expected to be at a low level and transient, and the fish are expected to make a full recovery.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Vertebrate development requires the co-ordinated growth, differentiation and movement of diverse tissues in time and space. While it is possible to model simple aspects of development in vitro, it is impossible to model the complex events that occur between tissues to form a fully functional embryo. Furthermore, pathogenic processes during disease, and attempts to treat them, involve many cell types and tissues and this cannot be recreated in vitro. It is for these reasons we must undertake experiments upon animals. Our species of choice

is the zebrafish as zebrafish are unregulated up to 5 days post-fertilisation and most of our experiments are performed on these unregulated animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To ensure minimum numbers are used, we will genotype fish and only keep those required. We will also store sperm when a genetically-altered line is not in active use and use in vitro fertilisation to rederive the line.

Refinement

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.

Refinement

We use the zebrafish model as a substantive body of work shows that developmental processes are highly conserved between vertebrates and because it is the vertebrate model with the lowest neurophysiological sensitivity. Thus, data derived from our models can be extrapolated to humans and other animals.
The protocols to be used are all standard methods in zebrafish research. With the exception of the minor surgery of fin tissues for genotyping which we hope to replace with swabbing in future, all of the protocols are non-surgical. When generating new disease models, we will use a small number of pioneer fish to set humane endpoints for further fish of the same genotype, and we will limit the length of time that fish will experience the disease.
Latest knowledge on analgesia will be applied.