Private & confidential: *Please be aware that the contents of this form may be made public resulting from the "Freedom of information Act". Personal details will not be released.*

G: NON-TECHNICAL SUMMARY (NTS)

Please attach the Non-technical Summary as generated by your application in ASPeL.

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 can 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.

Word limit; 1000 words

Project Title	The role and regulation of reactive oxygen species in development and regeneration
Key Words	Early embryonic development, Tissue repair and regeneration, Heart regeneration, Reactive oxygen species, Hydrogen peroxide
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;

No (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.

No (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):

The primary goal in regenerative medicine is to facilitate the replacement of aged, injured and diseased tissues with fully functional counterparts, thus extending the healthy life expectancy of our ageing population. My research group has been investigating the molecular and cellular mechanisms involved in tissue formation, repair and regeneration in Xenopus and zebrafish, two vertebrate model organisms with high regenerative capacity. We have discovered that appendage regeneration and embryonic development in these model organisms require sustained production of reactive oxygen species (ROS). ROS are natural by-products of metabolism, which, when produced at high levels, have traditionally been associated with degeneration and aging. Remarkably, our findings have shown that low, but sustained levels of ROS promote regeneration. We propose to extend these findings by addressing the following questions:

How is ROS production regulated during development and appendage regeneration, so that the right levels to promote regeneration are produced?

How does ROS promote regeneration?

We expect that answering these questions will pave the way towards the development of novel therapies, including the identification of novel pro-regenerative drugs, aimed at promoting tissue repair and regeneration in human patients, where regenerative potential is normally limiting.

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)?

Our aim is to identify the sources of ROS production and how they are regulated during development following tissue injury. How ROS promote regeneration will provide clues about how ROS production might be manipulated following injury or disease in humans, as a means of promoting regenerative healing in patients. We expect that, from these findings, we will identify one or more possible drugs or drug targets, which may improve tissue repair

and regeneration. That can then be explored in pre-clinical and clinical trials for their potential pro-healing/pro-regenerative effects in humans. The immediate beneficiaries of this work will be the fields of regenerative biology and regenerative medicine. However, the ultimate beneficiaries of these findings will be clinicians and eventually, patients who have suffered acute or chronic wounds or are suffering from degenerative diseases.

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

All proposed studies are to be performed on embryos and larvae from two frog species, *Xenopus laevis* and *Xenopus tropicalis*, and zebrafish embryos, larvae and adults. . These species have been chosen because they have remarkable abilities to repair and regenerate fully following injury. The approximate number of animals to be used under this licence will be approximately 40,000, of which most will be at the larval stages.

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 vast majority of procedures proposed in this license will not lead to any expected long-term adverse effects. The most often used protocol in the licence involves a simple injection of hormones, to induce ovulation and/or mating in Xenopus. The second most often used protocol under this licence will be the generation and maintenance of genetically modified frogs and fish. The remaining protocols involve transplantation of cells between embryos and larvae of fish and frogs, the treatment of larvae or adult fish and frogs with substances or heat pulses to alter gene expression, and the creation of wounds in larvae or adults in fish or frogs. On very rare occasions when adverse effects occur, the animals will be humanely killed.

Application of the 3Rs

Replacement

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

Replacement

The main objective of this project is to investigate the role and regulation of reactive oxygen species in development and regeneration. Most of the planned studies will be conducted in embryos or on isolated cells and tissues in culture. To study the formation, repair and regeneration of complex tissues and organs, it is necessary to perform work *in vivo*, as it is not possible to recreate fully the complex environment of the developing and regenerating tissues in culture. The complex multi-tissue events that occur during tissue repair and regeneration cannot currently be replicated fully, using tissue culture techniques alone.

Reduction

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

Reduction

Rigorous experimental design considerations will be employed in the conduct of all experiments to ensure that the minimum number of animals is used to reach meaningful conclusions. Overall numbers of animals required are based on initial sample size estimates. These numbers will be updated as more recent and relevant data becomes available.

Refinement

Explain the choice of animals and why the animal - 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

Our recent work has shown that early embryogenesis mimics many of the same mechanisms, in respect to the role of sustained ROS production in tissue regeneration---- and replicated tissue development. This has allowed us to refine many of our experiments in order to exploit these similarities, and thus, we will focus much of our future work investigating the basic science mechanisms of ROS in cultured early embryos. This has led to both **reduction** and **refinement** in our procedures.

In addition, frog embryos and larvae and fish larvae and adults are particularly well suited to this project because they have remarkable capacities to heal wounds quickly, without leaving scars. Complex tissues are regenerated within days or weeks following injury. This makes these organisms particularly useful in studying both the development of tissues and their repair following injury.