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)

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 project clearly using non-technical terms which will be understandable to 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 may 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 http://scienceandresearch.homeoffice.gov.uk/animal-research/).

(WORD LIMIT: 1000 WORDS)

Please complete the following:

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Understanding mechanisms of fibrosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Fibrosis, scar, therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td></td><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td></td><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td></td><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td></td><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td></td><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>Yes</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Fibrotic diseases are increasing and a major cause of morbidity and mortality worldwide. In some cases, end-stage diseases can be treated by transplantation; however, there is a huge shortage of donor organs; significant side-effects from immunosuppression; and focus on end-stage disease is too late. Urgent development of novel diagnostics to determine stage of disease and anti-fibrotic therapies are needed. This requires a better understanding of the underlying mechanisms of fibrosis to develop hypothesis based approaches to identify novel dynamic markers of disease and targeted strategies for therapeutic intervention. The aim of this project is to provide a greater understanding of the molecular mechanisms underlying chronic fibrotic diseases to instruct identification of novel diagnostic and therapeutic targets that can be used for patient benefit.
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)?	Fibrosis is a common step in the progression of the majority of chronic diseases. However, there are no approved anti-fibrotic drugs and diagnosis remains poor. Our work in this area has already uncovered novel mechanisms implicated in broad organ fibrosis that are currently under discussion with pharmaceutical companies as novel diagnostic / therapeutic strategies in fibrosis. There are clear implications for patient benefit and this has only been achieved by proof of principle using both in vitro and in vivo models of disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rat but more often mouse, particularly because of the ability to use genetically modified strains. Over a period of 5 years, with funding and staff / students working on these projects, I would expect breeding numbers to reach approximately 10,000 mice using several different genetic strains and for experimental protocols ~1,500 rats and 10,000-15,000 mice (a mix of wild type background and genetically modified animals).
In the context of what you propose to do to the animals, what are the expected adverse	In most instances, tissue from animals will be removed for studies in the laboratory. In some instances, animals will be treated with agents that

effects and the likely/expected level of severity? What will happen to the animals at the end?	cause fibrosis. Although transient discomfort may occur at the time of administration the animals appear normal soon afterwards. Similar to humans, animals can sustain fibrotic injury for a long period of time with no apparent symptoms. In the rare scenario that an animal shows signs of organ failure the animal will be put down to ensure the animal does not exceed the severity limits set out in the project. Some animals will undergo surgery to induce fibrosis, but these are not life-threatening procedures. Animals will suffer moderate adverse effects from this, which are similar to and primarily associated with the surgical procedure, the effects of which will be alleviated with pain-killing drugs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Despite progress in understanding the biology of fibrotic diseases, these discoveries have been unsuccessfully translated into the clinic. Fibrotic diseases are complex which develop and resolve over many weeks; involving the organ, immune system and cell-cell interactions. For this reason, it is not possible to study these events in isolation in an *in vitro* / ex vivo system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations performed based on an important component of fibrosis (collagen deposition) indicate 6 animals per group are required to analyse the fibrotic processes. For example our experience of biological variability shows fibrotic livers of 6 weeks CCl_4 treated rats have a mean collagen (hydroxyproline) content of 1.45 ± 0.25 (SD) mmol/g liver. Based on these data, accepting an 80% chance of detecting this difference at the level of p≥0.05, gives a sample size of $16/(1.74)^2$= 5.3 animals per group. Where possible we will make use of archived material and importantly make use human cells and tissue to reduce animal use. Through refining our technical skills, see below, we

	are also able to reduce animal numbers. Animal breeding will take into account the power calculations required for the experimental protocols.
3. Refinement Explain the choice of species 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.	In the case of cellular studies, particularly for liver fibrosis, we will use rats as this allows a greater analysis of the mechanisms associated with the disease process compared to mouse. However, for in vivo studies, mice will be necessary based on the use of genetically modified strains. To investigate the therapeutic potential of our findings in fibrotic disease in different organs from multiple etiologies, it is necessary to use more than one model of injury. We have chosen established models of organ fibrosis that have good comparison with human disease and have been refined over many years in labs worldwide. As evidence of limiting animal experimentation through refining our models, improved technical skills and post-operative care we have reduced the mortality of bile duct ligation from 30% to ~10% on our current liver fibrosis models. We will ensure similar refinement in all protocols (which are much less severe). As further refinement, and in agreement with our resident statistician, we will seek additional statistical assistance as required to refine experiments.
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Will the project be subject to Retrospective Assessment?[1]	Yes | No | Date due[3]:

[3] The retrospective assessment should be completed, agreed with the establishment AWERB, and submitted to the Home Office within 3 months of this date (or when the project terminates if earlier).