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">Immunopathology of experimental malaria infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Malaria, immune-pathology, immunity, brain</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 colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><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>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
</table></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)	The overall objective of this project is to identify the key pathways responsible for the development of immunopathology (i.e. tissue damage caused by the immune system) during malaria infection. It is becoming clear that the most severe complications of malaria infection, including cerebral malaria, respiratory distress and severe anaemia, are due, in part, to over activation of the host's immune response to the parasite. However, in the case of cerebral malaria, we still do not understand the interplay and interactions of the malaria parasite with cells of the immune system and with the endothelial cells that form the cerebral vasculature. Consequently, we still do not know the mechanism of vascular damage that leads to leakage, brain swelling and fatal outcome during the condition. Moreover, we do not know how anti-malarial drug therapy promotes recovery from the cerebral malaria syndrome in some individuals but not others, or the responses within the brain that resolve tissue damage and neuronal dysfunction following successful treatment of the condition. Crucially, in general, we do not understand how pro-inflammatory and anti-inflammatory immune responses develop and function during malaria infection, and why immunological homeostasis (i.e the balance between protective and pathogenic immune responses) sometimes fails to be achieved, leading to immunopathology. In particular, we have very limited knowledge of the behaviour of effector and regulatory T cells within tissues during malaria infection and how their interactions with other cells within the tissue determine their protective vs pathogenic activity.
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)?	This project may significantly advance our understanding of how protective and pathogenic immune responses form during malaria infection and will help to identify the specific events that lead to development of immunopathology, including cerebral malaria. These results should enable the development of targeted therapeutic and adjunct treatments for severe malarial disease, as well as identifying mechanisms through which to augment protective immune responses.
What species and approximate numbers of animals do you expect to use over what period of time?	The general project plan will involve infecting resistant (for example BALB/C) and susceptible (for example C57BL/6) strains of inbred and transgenic (for example IL-27R KO, IL-10-GFP reporter) mice with different species of *Plasmodium* parasites that cause specific types of immunopathology. We

	expect to use approximately 8600 mice during the course of this 5-year project, with 500 used or parasite maintenance / establishment; 5000 in different experimental designs; 3000 in GAA colony breeding and 100 for obtaining GAA animal tissue.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Depending upon the species and strain of malaria parasite and the strain of mice utilised, malaria infection may lead to mild, moderate or potentially severe suffering. *P. yoelii* XL, *P. berghei* NK65 and *P. berghei* ANKA parasites have the potential to cause severe suffering due to high parasitaemia and associated anaemia or cerebral pathology (the latter in case of *P. berghei* ANKA) in susceptible mouse strains. However, of the experiments involving infections that have the potential to cause severe suffering in animals, not all infections will be allowed to progress to the stage where severe suffering occurs. For example, animals may be euthanized at time points of infection preceding development of severe suffering to examine immune cell activation, regulation or the early events in pathogenesis of severe malaria infection. To help us to definitively address the importance of specific immunological pathways, we will employ different immunomodulatory techniques within the protocols, including localised and systemic administration of blocking and activating compounds, at precise stages of the experiment in relation to malaria infection. This will allow us to delineate the sequence of events that are necessary for the development or prevention of severe malarial disease and those important for parasite control. Most immunomodulatory techniques should not directly promote animal suffering. Irradiation with cell reconstitution will be performed under established protocols with appropriate monitoring to detect acute radiation sickness or reconstitution failure or more chronic radiation-induced suffering, such as dental complications. Anticipated effects due to anti-parasite chemotherapy will be monitored and animals exhibiting non-transient and non-recoverable levels of severe suffering will be quickly identified and euthanized. The final fate of animals on the licence will be either the transfer of animal to other project licences with the authority to receive animals that have undergone the procedures specified in the individual protocol or animals will be killed by exsanguination under terminal anaesthesia, a schedule one

	method, or following intravital (in animal) imaging under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We can only address the majority of our questions when a complete immune system is present in its normal anatomical and physiological configuration (for example within the spleen, the major site of immune priming and parasite killing during malaria infection), or when parasites and immune cells can interact with the complex architecture of the intact brain (leading to cerebral malaria); the use of animals is, to a significant extent, unavoidable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We calculate the required group size using data from pilot experiments, previous experience, and published work to ensure that we have sufficient power to detect a biologically relevant effect using as few animals as possible. *In vitro* assays, such as co cultures of parasites with endothelial cells or T cells with antigen presenting cells, will be utilised to replace animal experimentation where possible; however, animals will frequently be required to obtain materials for use in these *in vitro* experiments.
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.	Mice are the most appropriate species for this work as murine malaria infections are the most well-characterised of the various animal models and so much is known about their immune systems and all the reagents that we require are available. In terms of cerebral malaria, there is accumulating evidence that the nature of blood brain barrier disruption and the relative importance of the perturbation in driving cerebral pathology, are very similar in mice and in humans, validating the animal model for the study of the human condition. Animal suffering will be minimised by closely monitoring all animals in relation to a well-defined grading system and providing analgesia, when required.
For Office Use Only	
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).