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 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	Type 2 inflammation in health and disease
Key Words	Inflammation, Immunity, Parasitic infection, Allergy
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.

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.*

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

There is a global need to cope with parasitic worm (helminth) infection and allergies. Helminth infections affect a third of the world's human population and most mammals, while allergy is at epidemic levels in the developed world, and an increasing concern around the globe. This project aims to help our understanding of these conditions, as well as their relationship to one another.

The overarching aim of our research is to determine which cells are involved, and which mechanisms and pathways are used, to initiate, maintain and regulate a form of inflammation, called 'Type 2', found in parasitic worm (helminth) infection and allergic responses.

Specific objectives:

1. To define the function and importance of specialised immune cells called dendritic cells in initiation, maintenance and regulation of immunity and inflammation when the body is **challenged with substances** that alter immune responses

2. To define the function and importance of dendritic cells in initiation, maintenance and regulation of the immune response and inflammation against allergens and during **infection** with a helminth called *Schistosoma mansoni*

Our ultimate goal is identification of cellular and molecular targets for rational development of therapeutics.

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)?

The primary benefit of our work will be to discover new knowledge about the initiation, maintenance and regulation of Type 2 inflammation by identifying fundamental mechanisms that control inflammation. This will ultimately provide novel candidates for the development of therapeutics targeting cells or their products (in any inflammatory disease). It also has the potential to direct drug development.

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

The increased availability of genetically altered mice relevant to study of the immune system has allowed us to elevate and refine the questions we can address. Approximately 39,700 mice over 5 years will enable us to maintain genetically altered colonies (approximately 22,000) as well as investigate immune challenge with substances (8,000), and interrogate models of helminth infection (4,500), airway inflammation (4,200) and intestinal inflammation (1,000).

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?

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits.

Breeding and maintenance of genetically altered mice with specific deletions in immune function genes or transgenic expression of immune receptors are not expected to exhibit any harmful phenotype. However, as genetic alteration of key molecules or cell types can sometimes result in harmful phenotypes, it is our intention to only carry out experiments using such animals when absolutely necessary (1,000 over 5 years).

Our assessment of the importance of dendritic cells during Type 2 inflammation generated by the body in response to challenge with substances can result in local or systemic inflammation and pathology. This can manifest as weight loss, the involuntary bristling of fur, reduced spontaneous activity and reduced response to external stimuli. However, in most cases, only a small proportion of experimental animals will develop beyond mild symptoms to moderate severity limits.

Studies on immune and inflammatory aspects of infection with schistosomes will generate life cycle stages (eggs) of the parasite. From approximately 6 weeks post infection, schistosome infected mice may show swollen and distended abdomens, normally associated with weight gain. Some animals (up to 25% across experiments, depending on strain of mouse, infectious dose and time post-infection and, from our previous experience, approximately 10% over the lifespan of the licence) can suddenly succumb to infection from week 5 onwards, with no overt warning signs or evidence of suffering. However, the majority of our experiments will not involve strains of mice, doses or times post-infection that will lead to this level of disease severity. In most cases, only a small proportion of experimental animals will develop beyond mild symptoms.

In investigating lung inflammation by airway manipulation using established models, some animals (5-10%) may experience temporary (less than 24 hours) respiratory symptoms resulting in moderate severity limits. However, most experimental animals will not develop beyond mild symptoms.

The investigation of established models of inflammation of the small or large intestines generally result in moderate symptoms including weight loss, inactivity and loss of appetite, which can sometimes lead to bleeding and prolapse, resulting in moderate severity limits.

Some protocols will involve general procedures such as restraint, injection or use of anaesthesia. All of these provide the possibility of adverse effects, but none beyond moderate severity. All animals will be humanely killed at the end of each Protocol.

Application of the 3Rs

Replacement

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

Replacement

The mammalian immune system is highly complex, with many different cells and molecules working in combination to produce a co-ordinated response. Thus, the use of lower organisms such as Drosophila is not feasible, as they do not possess a complex immune system seen in mammals. Similarly, in vitro cell culture models cannot give an accurate reflection of the cellular and molecular complexity of a mammalian immune system. Thus, use of mammals is essential, with mice proving an invaluable tool in studying immunity and inflammation in the past 25 years.

Our research also depends on generating the egg stage of *Schistosoma mansoni* for use in subsequent studies. There is no alternative means of generating this life cycle stage other than in a mammalian host.

Reduction

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

Reduction

We reduce numbers of these animals in our experiments by using littermates where possible as controls. Increasingly, we are generating frozen embryos or sperm for later use. In many of our preliminary experiments, we generate primary cells from bone marrow isolated from only a few animals to test our hypotheses, before we embark on more extensive *in vivo* experiments that require use of larger numbers of animals. This approach also reduces the number of genetically altered animals that have to be bred to facilitate the research.

Our animal work is designed in consultation with statisticians and/or using the NC3Rs Experimental Design Assistant, in order to use the minimal possible animals in experimental groups that will still achieve significant results.

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 inbred laboratory mice and genetically altered mice for the vast majority of our research as they provide a range of refined approaches not available in any other species for investigation of immune cells and their products.

Mice are the most established animal model for study of the parasitic worm that we work with (schistosomes). Further, *Schistosoma mansoni* in mice is the most established model for human schistosomiasis. Parasite migration, maturation, egg deposition, and pathological consequences of infection in the mouse are similar to the processes in humans. A proportion of *Schistosoma mansoni* infected mice may show signs associated with hepatosplenic disease from approximately 6 weeks post infection. Doses are carefully adjusted to minimise adverse effects.

Sensitisation and challenge of mice with allergens or allergen-loaded cells generates airway inflammation that is an accepted model for studying mechanisms underlying human asthma. Similarly, dextran sodium sulphate (DSS), methotrexate or cell transfer provide accepted models of intestinal inflammation that are the foundation of innovative research into colitis and inflammatory bowel disease in humans.

We are constantly assessing and refining our methods to give the best possible scientific approach coupled with the minimal severity and numbers of animals used. In all studies, animals will be closely monitored and should any unreasonable or unexpected loss in condition be observed, the animals will be humanely killed.