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:

Project Title (max. 50 characters)	PKCα and vascular calcification in kidney dysfunction.	
Key Words (max. 5 words)	PKCα Vascular calcification Chronic kidney disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals[1]

[1] 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)	Bone-like calcium deposits are found in the walls of blood vessels of over 80% of patients with chronic kidney disease (CKD), diabetes and atherosclerosis. These deposits contribute to a reduction in the quality of life and premature death. Despite the serious nature of this condition there is no effective treatment available to patients. Extensive studies performed using isolated cells in culture have shown that an enzyme, protein kinase Cα (PKCα), is a regulator of calcium deposition in blood vessels (vascular calcification). Before we can transfer these basic observations into a potential therapy, we need to confirm that PKCα regulates vascular calcification in the more complex whole body setting. The aim of this project is to establish whether PKCα regulates the vascular calcification that occurs as a result of CKD. If PKCα is shown to be a regulator of vascular calcification we aim to develop therapeutic interventions which target PKCα with a view to slowing down or preventing vascular calcification.
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 fatal consequences of vascular calcification for the large and growing number of patients with CKD, and the potential of this study to identify a novel drug target for this pathology, make this project necessary and worthwhile. In the short-term, we will determine whether PKCα plays a role in the development of vascular calcification that occurs as a result of renal dysfunction. This information would be a major advancement of our current knowledge of the biological regulation of vascular calcification in chronic kidney disease. In the longer term, the knowledge gained from this project could justify larger scale animal trials of drugs that modulate PKCα, with the aim of moving onto human clinical trials in the future.

What species and approximate numbers of animals do you expect to use over what period of time?	We have requested authority to perform experiments under this licence for the standard 5 year period. We will use mice for the planned experiments. Some of these mice will be normal animals that will be used as controls against which to compare differences. The remaining mice will have been genetically modified such that they are unable to make PKCα. We will study both male and female mice, unless it becomes apparent that one of the sexes is un-responsive to the experimental manipulation in which case we will discontinue using that sex. In order to set up a breeding colony to supply sufficient mice lacking PKCα for the experimental work we will require 700 mice. In order to determine how long we need to treat mice with a diet enriched with phosphate in order to induce vascular calcification, we will perform a pilot study using a total of 48 mice. Once we have established the most appropriate time scale, we will conduct the main experiment using both normal animals and mice lacking PKCα, potentially of both sexes. Based on our previous experience of this experimental approach we have calculated that we will need up to 17 mice per group to detect meaningful differences in the degree of calcification. We will also need a further 10-15 mice per group for follow up experiments designed to identify the underlying molecular mechanisms. These experiments will require up to 152 mice if we study both sexes. The total number of mice that we are likely to require for breeding = 700 and the total number of mice for experimental purposes = 200.

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?	The severity limit of this project is moderate, because animals will undergo invasive surgery and then be allowed to recover. The surgery itself carries a small risk of acute renal failure (< 5% in our hands). Post-operative pain will be prevented through the use of analgesics. The combination of renal reduction surgery and a high phosphate diet is designed to induce accelerated vascular calcification which would eventually lead to heart attack or a stroke. However the time-frame of the experiment is such that mice will not experience these effects. Mice are expected to develop chronic kidney disease which is ultimately fatal. However once again this will not occur within the time-frame of the experiment. In order to monitor the health of the animals we will periodically take blood samples. Mice will experience transient discomfort from a needle prick required to collect blood. At the end of the experiments all mice will be killed by a Schedule 1 method or under terminal anaesthesia. An imaging technique (computerised tomography – CT scanning) will then be used in order to generate a 3-D picture of the main blood vessel, the aorta, in order to map the distribution and quantify calcium deposits in the wall.

Application of the 3Rs				
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	CKD leads to complex changes in physiological function, both within the diseased kidney and in the body as a whole. Many of these changes are not understood fully and so cannot be reproduced using cell culture or computer modelling. However it is precisely these systemic changes that result in vascular calcification. Consequently, it is necessary to study the changes that occur in blood vessels in the intact animal in order to understand what is happening as CKD progresses. This leaves us with no viable alternative to the use of animals for the proposed project.			
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have designed our experiments so that we are able to gain the most possible information from each individual animal. We have performed sample size calculations based on our own experience with the animal model and that of others using similar approaches so that we know how many mice will be required to give statistically meaningful results. We have built in a pilot study to establish whether both sexes need to be studied.			
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.	We will use mice in this study because they share similar physiology with humans. They can also be manipulated genetically with ease, allowing us to study our target molecule of interest. We will use a model that involves reducing the mass of the kidneys and feeding a high phosphate diet in order to induce accelerated vascular calcification. The surgical approach itself is recognized as being a good model of human CKD. The addition of a high phosphate diet means that mice will develop the pathology of interest quickly so that they do not have to be kept for an extended period of time. We have built in a pilot study to determine how long we need to feed mice a high phosphate diet in order to produce a detectable change in vascular calcification so that the length of the experiment is optimised. Clearly defined humane endpoints have been built in to the project to ensure that animals do not suffer unnecessarily.			
For Office Use Only				
Will the project be subject to Retrospective Assessment?[1]	Yes	No	Date due:	