Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 11

Projects with a primary purpose of: Translational and Applied Research – Human Urogenital and Reproductive Disorders

Project Titles and keywords

1. **The pathophysiology of the overactive bladder**
    - Overactive bladder, pathophysiology, cystometry. PDE5 inhibitors

2. **Mechanisms in very early embryos**
    - Meiosis, reprogramming, mouse, micromanipulation

3. **Designing a localised drug delivery system to treat endometriosis**
    - Endometriosis, gynaecological, infertility

4. **A study to identify new treatments for renal fibrosis**
    - Kidney, fibrosis, CKD

5. **Role of intrarenal humoral control of renal function**
    - Kidney, physiology, transport, hypertension, diabetes

Project 1	**The pathophysiology of the overactive bladder**	
Key Words (max. 5 words)	Overactive bladder, pathophysiology, cystometry. PDE5 inhibitors	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To measure the reduction of overactive bladder behaviour by treatment with PDE5 inhibitors and to characterise the mode of action whereby these agents mediate this action.	
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)?	Overactive bladder activity remains an extremely prevalent condition in the general population. The incidence increases markedly with age, which is of direct relevance in the ageing population. It dramatically decreases the quality of life for sufferers; it directly increases patient morbidity through consequential problems such as increased risk of falls and skeletal injuries due to the need to toilet in the night-time. The cost to the NHS of managing OAB, especially in the community, is enormous — amounting to about 3% of total healthcare costs. Currently pharmaceutical therapy is the mainstay of treatment, but the available drugs are often ineffective or have significant side-effects that limit severely patient compliance. PDE5 inhibitors offer a novel	

	route of treatment but their mode of action remains unknown. These agents are safe to use for other medical conditions, such as erectile dysfunction in men, but their efficacy to treat OAB conditions and how they achieve this end are not known. This project will potentially characterise a novel drug target to manage this prevalent, distressing and expensive condition.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used for the project. OAB will be generated numbers of animals do you using well-validated procedures. Furthermore there is expect to use over what period of substantial literature concerning the physiology and pathophysiology of lower urinary tract function in these animals so thai baseline recordings (before use of PDE5 inhibitors) may be compared to these data. For the five-year duration of the project it is estimated that the per annum use will be 120 mice
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?	OAB will be generated in these animals by two procedures: i) spinal cord transection; ii) bladder instillation of irritants. These procedures are of moderate severity. Spinal cord transection will reduce hind-limb mobility and urinary retention — the latter will be managed by manual expression of urine with gentle abdominal compression. Irritant instillation will not produce general pain of discomfort at the concentrations used. The animals will be humanely euthanised about a week after an overactive bladder condition has been generated. Tissues will be removed for physiological, biochemical and molecular characterisation in the presence and absence of PDE inhibitors.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project aims to characterise the effects of a class of agents (PDE5 inhibitors) on the overactive bladder (OAB), an extremely prevalent and distressing symptomatic condition in men and women, which has several clinical origins. These agents were developed to manage sexual dysfunction conditions in men, but there is anecdotal evidence from patients that they may also minimise OAB symptoms. However, their

	mode of action is unknown and it is not known if they would work for all OAB patients, if they work on the bladder of females, and what is their site of action (e.g. bladder muscles or nerves that supply the bladder. This study will investigate their mode of action, using two well-validated animal models of overactive bladders that have close parallels to human conditions: spinal cord injury to produce a so-called neurogeriic overactive bladder; and bladder urgency generated by intravesical cystitis using an irritant (e.g. acrolein). Cultured cells may not be used as the bladder contains many different cell types. Spontaneous bladder contractions associated with OAB, result from a complex interaction between these different cells, of which several may be targets for PDE5 inhibitors and thus requires investigation within the native tissue. Likewise it is not fruitful to use computer-based, computational approaches as the basic principles by which the different cell types interact to generate OAB behaviour is equally not understood. Human bladder samples from patients with and without OAB can eventually be probed for PDE5-inhibitor targets. However, the sole use of human tissue would not allow us to achieve the aims of this project for several reasons: patients who donate tissue samples come from a heterogeneous population often with co-morbidities that confound interpretation of data; obtaining sufficient material will be very difficult; tissue samples that contain some of the cell types we which to investigate (e.g. afferent nerves) do not survive well the biopsy-taking procedures used in humans, and integrated models with connections to the nervous system are not possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments are subject to power calculations to keep the required number of experiments to a minimum but concordant with producing meaningful scientific data. The applicant has considerable experience with all the techniques to be used for the experimental phases of the study, which will minimise

	any wastage of tissue (and hence number of animals) to achieve scientific data. In addition, the applicant has experience with the procedures listed above, as well as animal monitoring in the post- surgical phase which will also minimise the number of animals that will be used.
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 have been chosen as the applicant has considerable experience with their use in studying bladder physiology and pathology and there is also a very large body of peer- reviewed literature concerning their use in this context. This means that the data obtained in this project can be placed in the context of a very large body of similar data and so makes evaluation of the specific data from this project easier to evaluate. Experiments will be done using a preparation where only the most primitive region of the brain (the brainstem) is connected to the bladder — a decerebrate preparation - to exclude any possibility of pain or sensation. Connection to the brainstem' is essential as this region exerts an essential control over bladder function and the agents to be studied (PGE5 inhibitors) could act at several areas within the bladder-brainstem axis. With respect to minimising welfare costs, the applicant has considerable experience in handling, carrying out procedures and health-monitoring mice for adverse events. The applicant will ensure that all personnel engaged in the project learn these skills as effectively as possible, to ensure than an excellent standard of animal welfare is embedded in the group.

<table>
<tr><td>Project 2</td><td colspan="2">MECHANISMS IN VERY EARLY EMBRYOS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">meiosis, reprogramming, mouse, micromanipulation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
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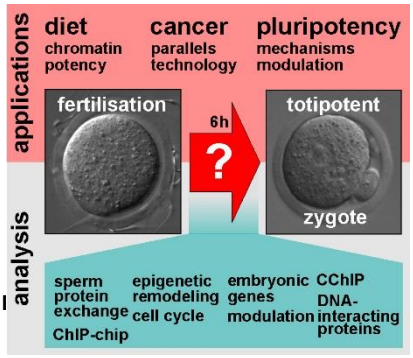
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">We wish to understand how a sperm and an egg (the gametes) transform into an embryo. In particular, what critical steps and interactions immediately after mammalian gametes combine in fertilisation are needed to establish a 1-cell embryo?

This egg-to-embryo transformation (Fig. 4) represents a profound programmed cellular change, from assured cell death to the formation of a cell that can give rise to an entire individual. Although this is a fundamental developmental process, little is known about it.

Once inside the egg, the fertilising sperm is unpackaged by unknown processes. Concomitantly, the sperm 'informs' the egg that it has arrived and triggers it to resume cell cycle.</td></tr>
</table>

	This overlaps with a phase in which the relatively denuded paternal genome becomes re-clothed with maternally-derived histones and other proteins that are further remodelled to generate fully functional embryonic chromatin. All of these processes are highly integrated within the very early embryo, but very little is known about how, or what the underlying mechanisms are.
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)?	Understanding the mechanisms of very early embryogenesis promises to reveal parallel pathways that initiate cancer - at least we will be able to evaluate the extent to which such parallels exist. Moreover, a detailed molecular model of how the very early embryo is formed should inform protocols for induced pluripotent stem (iPS) cell generation, which today are unsafe and inefficient and hold little realistic prospect of approval for clinical use. How? Because our work concerns a natural process (fertilisation) that is highly (~100%) efficient; applying the underlying mechanisms and molecules promises to render iPS cell induction safer and more efficient. Only by understanding these natural processes will we be able to manipulate cellular potency safely for applications in regenerative medicine.
What species and approximate numbers of animals do you expect to use over what period of time?	This work requires the manipulation of gametes, for which there is no acceptable alternative *in vitro* source to animals. Because we wish our studies to be applicable to humans and because there are clear differences between mammals and other models we generally work on the mouse, whose gamete and developmental biology and genetics are tractable. This is not the case for the main *Xenopus* model (*X. laevis*) which is tetraploid and differs in multiple aspects of oocyte activation and early development, including Ca^{2+} release dynamics, requirement of protein synthesis, spindle role and the onset of embryonic transcription. Discrepancies have recently been documented between the cytostatic factor, Emi2, in *Xenopus* and *Mus*. The mouse is especially important as a model system for mammals because we wish our results to impact human clinical medicine; a senior goal of this work is the reduction of disease-induced human suffering. Moreover, there is a limit to which meiosis can be modelled in mitotic cells cultured *in vitro*, because the two are dissimilar and no alternative to embryo transfer into a recipient mother for the development of preimplantation embryos to term. This work requires up to 5,000 animals per year, mainly for the

	production of eggs and preimplantation embryos, but also for the generation of genetically altered animals (GAA).
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?	In an overwhelming majority of cases, no adverse effects are expected. All of the methods are standard. Phenotypes cannot be predicted and therefore all GAA are carefully monitored for signs of sickness in consultation with the Named Veterinary Surgeon. Where he or she thinks that the animals are in prolonged moderate discomfort, they will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This work needs to use animals because there is no alternative source of the cells we use - sperm and eggs - and they have to be collected freshly from mice. There is at present no system for producing oocytes *via* tissue culture *in vitro* or by any other method than animals themselves. Reports of oocyte-like cells produced *in vitro* have not in fact generated oocytes; no offspring have arisen from them, they differ from *in vivo*-derived oocytes morphologically and it is not known whether they express key markers. Moreover, they have been generated at low efficiency, inconsistently and with difficulty. Similar constraints are placed on the source of spermatozoa: they cannot be derived by *in vitro* culture. We need to evaluate post-implantation development, for which there is no *ex vivo* alternative to embryo transfer. The mouse is the only tractable model for mammals owing in particular to the optimisation of its genetics and embryology. Thus, opportunities for replacement are tightly constrained and clearly, our experiments would be completely unethical in humans. Some workers argue that *Xenopus* is a suitable replacement model system for the mouse in the study of human biology. We emphatically reject this dangerous assertion. There are similarities between frogs and mammals, of course (as there are between *E.coli* and mammals), but there are also marked differences, and they are not possible to predict for any given feature. For example, there are no adult frogs cloned from adult-derived cells, even though frog cloning is ~35 years older than cloning mammals; these and other profound biological differences

	have been reviewed. It is vital in our work that we know that the gametes we use are normal fully functional in all respects because we are investigating fundamental mechanisms. Without this assurance, the experiments may be a waste. This said, we shall continue regularly to consult the internet for alternative sources of mouse gametes. We expect that a corollary of our work will be to identify natural mediators of pluripotency that can be harnessed to improve *in vitro* models that in promise in some cases to replace *in vivo* ones.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Standard superovulation maximizes the yield (reliably yielding ~20 per female), minimizing animal numbers. In my 19-year experience, an average of 40~50 female B6D2F1 females are required for superovulation (Protocol 01) per worker per week performing micromanipulation, embryology and molecular experiments. Embryo transfer (protocol 02) is a standardised procedure whose efficiency we will maximise by placing good embryos in the hands of well-trained staff. Transfers are essential for monitoring full developmental potential of embryos, for example in the generation of transgenic offspring (Protocol 04). We will identify efficient stud males (Protocol 03) to minimise matings. For BrdU labeling experiments (Protocol 06), the number of oocytes injected with BrdU-labelled sperm will be maximised per animal by superovulation where superovulation is possible. Treatments often yield binary outcomes such as developmental activation, which either occurs or does not and is carefully controlled for. Care is also taken to avoid bias by routine blinding, in which Worker 1 records the outcome of coded experiment of Worker 2. Robust measurements in our work are subject to known and unknown parameters that affect outcomes and planning. Knock-down analysis serves as an example. The consequences of depleting target mRNAs will be determined in oocytes and early embryos using siRNAs or morpholinos (MOs). For a given target, test siRNA is injected (with sperm) into 50~200 oocytes. The number depends on the efficacy of the siRNA (which can be zero), its kinetics (if too slow, oocyte aging becomes a factor), protein turn-over and antibody affinity. Tests are in parallel with control siRNAs (or MOs). For SDS-PAGE, ~100 embryos/lane may suffice (although this value is far higher for some antibodies), but even high-end mm requires 1 worker-day to achieve this reliably, so samples may be archived at -80. 0~40% embryos perish after manipulation and ~30% of experiments fail to produce

	useable samples. After immunoblotting, if the control target protein band intensity decreases from 100 to 60 (an above-average decline) with an sd of 30 over 3 experiments, the statistical power is 74.7%, rising to 90.9% with 5 experiments. All experiments will be validated with at least two siRNAs/target and in some cases, multiple antibodies, for example if the candidate protein exists in multiple forms (common in early oocytes and embryos). Published work optimises RNAi in oocytes and transgenesis and editing studies minimize animal numbers by producing improved models containing fluorescently-labelled gamete proteins and utilising a highly efficient method developed by us or by genome editing via a highly efficient method also developed by us. This work promises novel and improved methods of genome manipulation in mice and larger mammals, and it can realistically be hoped that they will reduce requisite numbers of both. Where suitable lines already exist, animals will be obtained from the relevant supplier or re-derived by embryo transfer (Protocol 2) from our previous work.
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.	The mouse as a standard model for gamete biology and embryology, including for humans; no others combine high-through-put intact oocyte micromanipulation, maturation *in vitro*, genetics, embryology (*in vitro* and term development), transgenesis and imaging. Use of piezo-actuated microinjection will ensure that the most data is obtained from biological samples. One application of this work is to improve iPS cell derivation, resulting in a long-term decrease in the need for animal research in this area.

<table>
<tr><td>Project 3</td><td colspan="3">Designing a localised drug delivery system to treat endometriosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Endometriosis, gynaecological, infertility</td></tr>
<tr><td>Expected duration of the project (yrs.)</td><td colspan="3">Five</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Endometriosis is a gynaecological disorder which affects 10% of women of reproductive age and causes symptoms of chronic pelvic pain and infertility. Current treatments are associated with an unacceptable level of side-effects or impairment to fertility. New management approaches arise from understanding the mechanisms that enable endometrial cells to survive in unwanted locations. We aim to gain improved understanding of the mechanisms involved in endometrial cell implantation by different stages of disease progression. We also aim to investigate novel treatment strategies by using an animal model which is well validated against the human disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">It is evident that there is a need for further refinement of preclinical animal models to ensure maximum resemblance to human disease. We</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	have to design our preclinical studies in such a way that enables the most rigorous drug efficacy testing to enable clinicians to make informed decisions regarding the reliability of transferring the therapeutic to humans. Benefits of this work are: 1) advancing our scientific understanding of mechanisms which aid survival of endometrial cells at unwanted locations 2) refinement of animal models enabling more robust translation to clinical research 3) validation of the animal model to human disease 4) potential validation of imaging techniques applicable to endometriosis models 5) proof-of-concept studies that drive new targets towards clinical development
What species and approximate numbers of animals do you expect to use over what period of time?	Approx. 1500 female mice over 5 years
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?	In a typical experiment we will surgically induce endometriosis in mice then treat them with various therapeutic approaches and monitor endometrial lesion response by direct calliper measurements or imaging techniques. Developing these models and using them for drug testing is labelled moderate severity. Endometriosis is induced by transferring non-fluorescent/ fluorescent endometrial tissue from donor animals to recipient mice via surgical procedure. Adverse effects due to endometriosis initiation are rare, but could include infection, pain or introduction of mouse pathogens. These effects are all countered by good aseptic technique, use of analgesics and screening of murine cell lines to ensure no pathogens are present. Surgically

	introducing endometrial tissue will control where lesions will develop to minimise the probability of them disrupting other biological systems and affecting animal behaviour. Imaging is incorporated to monitor endometrial lesions in a non-invasive manner and to provide a reliable tool for assessing treatment efficacy. Pilot studies will be used to determine model progression and identify early indicators of decline in well-being that can be used as end- points for subsequent studies (e.g. general loss of condition or weight). Overall, wellbeing is monitored using a Health Score Sheet recording system that uses measures such as weight, appearance and behaviour in addition to evaluation of lesion burden/appearance to give an assessment of mouse welfare. This enables early interventions before substantial deterioration is observed and allows the accrual of information used to improve the overall process in subsequent studies (e.g. recognising the timeframe over which endometrial lesions develop and increasing frequency of monitoring accordingly, identification of clear end-points). Through recording and monitoring we endeavour to ensure adverse events are minimised and moderate. In all cases the animals are culled at experimental endpoint.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot yet fully model the complex peritoneal environment, signalling processes or cellular interactions which are crucial for endometriosis development in humans. Practically and ethically we cannot test in the clinical setting and therefore must use species with physiology that best represents what we would expect to find in the human diseased state. Before initiating *in vivo* studies we will undertake comprehensive studies *in vitro* using endometrial

	cell cultures derived from mice and humans to ensure the clinical validity of the model. Drug efficacy studies will be completed in these cell systems coupled with *in silico* evaluations of drug/target interactions and pharmacokinetics to enable informed judgements regarding which therapeutics should be tested.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce inherent variability by using adult, same sex, age matched animals. We treat animals bearing endometrial lesions of equivalent size, which markedly improves the uniformity of response, thereby requiring fewer mice. We will use a randomised design with group sizes determined by power analysis using freely available software (SISA, http://www.quantitativeskills.com/sisa/). Correspondence with expert biostatisticians and researchers who have developed an animal model of endometriosis at the University also contributes to fewer animals. Where appropriate imaging techniques will be employed to enable multiple assessments to be made in the same mouse, pre and post therapy. Often applying a sequential study design in this case as it may not be possible to image all mice required in a single imaging session. The initiation of endometriosis is staggered over time, with generally 4 mice imaged per day. This is repeated until the experimental group sizes are achieved. Archived imaging data is used to refine sample size calculations enabling a reduction in group sizes.
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.	Although primates spontaneously develop endometriosis identically to humans the least sentient animal sufficient for this research will be used. Mice will be artificially induced with endometriosis because they develop lesions which most closely resemble human disease compared to other rodent species. They have the lowest neurophysiologic sensitivity in which well-characterised models of endometriosis already exist. Correlative studies reveal mice and women share similar genetic alterations and sensitivity to

	hormones involved in endometriosis, indicating the potential for inter-species translation of results. To minimise welfare costs we closely monitor mouse wellbeing and identify appropriate intervention points from pilot studies. Following surgery animals will be closely monitored and individually housed in temperature controlled incubators with more accessible food until fully recovered. Imaging techniques will be employed to visualise changes over time and determine when best to analyse by histology.

Project 4	**A study to identify new treatments for renal fibrosis**	
Key Words (max. 5 words)	Kidney, fibrosis, CKD	
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
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project licence is to identify new potential therapeutic targets for the treatment of chronic and progressive kidney disease. End-stage renal failure requiring dialysis affects 35,000 people in the UK and has a mean five-year survival of 87% in 18-34 year olds, falling to 29% in 55-65 year olds making its prognosis worse than most of the common cancers. Although kidney injury can be initiated by a number of unrelated conditions such as diabetes, hypertension, once started, it is the independent and self-perpetuating process of fibrosis and scarring that eventually damages the kidney causing them to fail. Fibrosis (scarring) is a particularly difficult process to treat as it has many diverse causes and multiple contributing factors and currently there are no specific therapies for slowing its progression. Both dialysis and transplantation, whilst effective, are extremely costly therapies, highly invasive and often distressing for individuals. For this reason part 2 of the UK Government's National	

	Service Framework for Renal Services has highlighted the need to “support NHS organisations in the prevention of chronic kidney disease in people at risk” and to “develop strategies for slowing down the progression of the disease”. During the development of chronic kidney disease, specialized cells within the kidney called fibroblasts and mesangial cells begin to multiply and to produce excessive amounts of scar tissue know as matrix. This matrix gradually overtakes the normal architecture of the kidney and obliterates the normal, functional cells. As part of our aim to identify new treatment targets we have been studying these cells in non animal-based, in vitro (laboratory) experiments to be able to understand the signalling pathways within them that are switched on when they start to multiply and produce matrix. Using these cell-culture experiments we are able to screen potential targets and new treatments for their ability to stop cell growth or prevent matrix production. In this way we minimise the number of animals we need to use as we only use animals when cell culture has shown promising results. The process of fibrosis is, however, complicated. Non animal-based models, whilst excellent for screening potential treatments for fibrosis, are not adequate to assess final impact or potential unwanted effects such as poor wound healing. Humans cannot be used in the first instance as the experiments involve new and novel compounds, not previously given to humans. Whilst we expect a number of them to be effective, not all will be and the potential to cause harm means this form of investigation is unethical in people without prior animal screening.
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 find new treatments designed to prevent or slow progression of chronic kidney disease. Not only will this reduce the number of patients on dialysis treatment in the future, but will also reduce the total disease burden in the country for other conditions related to chronic kidney disease (CKD), such as heart disease.. Patients with CKD are at increased risk of developing cardiovascular disease

	and patients with CKD most commonly die from heart disease. Reducing the burden of CKD in the population could also therefore reduce the overall burden of cardiovascular disease. Anti-fibrotic agents are also likely to be effective in treating other chronic diseases. Liver failure, heart failure and lung failure are all associated with fibrosis in the effected organs and could benefit from any new treatments that reach clinical practice as a result of this project licence, without the need for further animal experimentation.
What species and approximate numbers of animals do you expect to use over what period of time?	We are intending to use both rats and mice for our experiments. We have previously established reproducible and effective models of renal fibrosis and renal failure in both of these species. Rodents develop kidney scarring and failure in a very similar way to humans and many models mimic the human condition closely. We will minimise the number of animals we use whenever possible but we estimate that we will use approximately 830 mice and 450 rats over the five-year period.
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 overall severity classification for our experiments is 'moderate' although some are 'mild'. This licence contains 4 animal models of kidney disease: 1. Kidney disease caused by obstructing the flow of urine from kidney to bladder by blocking the ureter (this is similar to what can happen in men with prostate disease). 2. Causing kidney damage with drugs or antibodies that affect the kidneys, 3. Inducing diabetes in the animal to study diabetic kidney disease and 4. Causing kidney damaging by temporally reducing the blood supply to the kidney as can happen following trauma or surgery in humans. Some of the experiments we intend to undertake will involve performing surgery on animals. During surgery they will be under general anaesthesia and post-operatively they will receive adequate pain relief. They are expected to recover well following all surgical procedures. Animals will also undergo blood tests, imaging and be placed in metabolism cages for

	up 12 hours to allow us to collect their urine. The models described above are designed to produce kidney scarring and fibrosis. As in humans, up to 80% of the kidney tissue can be scarred resulting in moderate renal failure without the animal exhibiting any signs of ill health. However, should the damage get worse, the animals will lose their appetite, feel generally unwell, lose weight and possibly retain excess fluid in the abdomen. If we see signs indicating that the animals are deteriorating, they will be promptly and humanely killed. The animals that are made diabetic will experience all the symptoms that patients experience such as increased thirst, weight loss and tiredness. We will keep the animals' blood sugars at a level to minimise these symptoms by using insulin, but if they deteriorate they will also be humanely killed. At the end of the experiments all animals that are not being kept for breeding purposes will be humanely killed and their tissues will be stored for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Fibrosis is a complicated disease process that cannot be adequately modelled in cell culture. Although the majority of our screening protocols are carried out in cells, the final stage of testing efficacy and unwanted side-effects needs to be completed in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have a long track record in animal experimentation and have developed ways of designing experiments that minimises the number of animals used, whilst maximising scientific data obtained. We have built upon our previous experience extensively, ensuring experiments do not need to be repeated. We have a clear understanding of what we need to measure and how many animals we need to use to detect a significant effect of any treatment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	There are a number of animal models of renal disease in existence. We have refined many of these over the last 5 years to ensure that we get reproducible results, in the shortest time period possible, with the minimum of suffering. We are

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	experienced in identifying animals who exhibit signs of renal failure and can therefore humanely kill them at the earliest stage possible.

Project 5	**Role of intrarenal humoral control of renal function**	
Key Words (max. 5 words)	Kidney, physiology, transport, hypertension, diabetes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	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
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		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In the kidneys, blood is filtered into the lumen of tubular structures called nephrons, the functional units of the kidneys. The nephrons reabsorb (take back) into the blood everything that is not to be excreted. This project focuses on every section of the nephron – the proximal tubule, the loop of Henle and distal tubule – that reabsorb a large proportion of the filtered sodium and potassium (the distal tubule secretes this cation) and almost all of the filtered glucose, and the final section of the nephron – the collecting duct – which is responsible for the fine control of sodium and water excretion. It is already established that hormones circulating in the blood influence the reabsorption of sodium, potassium and water, but it is now becoming clear that other chemicals, present within the lumen of the nephron, can also make an important regulatory contribution. This project will determine how locally released	

	chemicals (nucleotides, e.g. ATP, which act on specific [P2] receptors) and certain enzymes within the lumen can affect reabsorption in the loop of Henle and/or collecting duct and thereby influence overall excretion. The aim of our project will also be to determine the effects of type-1 (T1DM) and type-2 (T2DM) diabetes mellitus on renal proximal glucose reabsorption transport in vivo, which has not previously been characterised. We will also examine the effect of T1DM and T2DM on glucose transport in the final section of the nephron, and investigate whether tubular sweet taste receptor stimulation contributes to the observed changes in glucose transport.
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)?	By defining the local regulation of ion and solute transport along the nephron in normal rodent kidney, and in some specific disease models, we believe we can not only advance our knowledge of renal tubular transport physiology and pathophysiology, but also identify potential therapeutic targets. Nephrology as a specialty has had few new and well-understood drug therapies since the discovery development of diuretics. Renal tubular transport regulation is key to normal fluid and electrolyte balance in health and disease, including, for example, hypertension and diabetes.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 200 mice and 650 rats will be used over the 5-year period. Approximately 2500 mice will be used for breeding and maintaining genetically altered and mutant rodents over the 5-year period.
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 genetically and non-recoverable surgically altered models that will be used in this proposal have all been characterised in detail by a number of different research groups. In addition the applicant has experience of all the surgical techniques and animal models described in this proposal. Based on our previous studies, animals undergoing induction of diabetes using streptozotocin may show signs of diabetes with increased eating, urine formation

	(polyuria), and water drinking (thirst); however dietary manipulation, as envisaged, causes no obvious adverse effects. The possibility that the streptozotocin injection (via the tail vein) to induce type 1 diabetes may cause infection at that site of injection is minimised by using aseptic conditions during the procedure. The techniques described in this proposal are of mild-to-moderate severity. All animals undergoing a procedure will be weighed at regular intervals and any animal showing loss of weight greater than 10%, associated with other clinical signs of distress, such as diarrhoea, excessive urination or drinking, decreased food intake, or failure to self-groom, will be killed by Schedule I method. In the event that any animal shows signs beyond those described in this licence, the NVS will be contacted for advice and treatment, and if advised, killed by Schedule 1 method (cervical dislocation or anaesthetic overdose). All experiments, aside from dietary manipulation, metabolic cage housing, and induction of type 1 diabetes by streptozotocin, will be done under deep anaesthesia from which the animal will not recover consciousness, thereby minimising animal suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An important aspect of the proposed work is to establish how the kidneys function to regulate levels of glucose, sodium, potassium and water in the body. This is likely to involve the releasing factors into the blood or urine that circulate around the body and act on the kidney to adjust transport. High-blood pressure, diabetes and chronic renal failure are likely to affect these interactions. These complex interactions cannot be reproduced under cell culture conditions making this method inappropriate for use in this project. In addition, cell lines available often have different transport and structural properties than native tissue.
2. Reduction	Wherever possible the maximum number of samples

Explain how you will assure the use of minimum numbers of animals	will be taken from each animal (blood, urine, and different organs) so as to collect as much information as possible for the present project or future studies This approach not only reduces animal use, but also allows more detailed and precise interpretation of the data without increasing the suffering of the animal. A meticulous work (storage, labelling and analyses of samples) is also essential for not having to repeat the experiments and thus reduce the number of animals used. Animal group sizes are based on the numbers needed to achieve a statistically significant difference for the key measures to be made, but wherever possible, blood and tissue samples will be collected in such a way as to maximise the experimental information gained from each animal under a given experimental condition.
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 most instances the rat is the animal of choice for these studies since there is extensive information available on renal sodium and glucose transport. In addition, surgery and chemical induction of diabetes is generally easier and more reproducible in rats. However, genetically modified mice, such as those lacking the gene for specific transporter proteins will also be used under this licence. The protocols used in this proposal have been designed to be the most refined possible, using the minimum number of animals, to provide statistically satisfactory results. All types of diabetic animals as well as food pellets will be weighed regularly. More frequent feeding and watering will occur. Cages will be cleaned, and bedding and nesting materials replaced more frequently for the duration of the procedure, and animals will have ready access to water ad libitum. Diabetics will be pair housed, preferably with a diabetic control. Control animals will act as a ‘cage buddy’, assisting their diabetic partner with grooming and also providing contact warmth and comfort. Confinement in metabolic cages is a stressful experience for animals. It will be kept to a minimum,

	but for some mice may include the full 3-week period. When possible, where the purpose of using a metabolism cage is not frustrated, the cage environment will be enriched with eg, plastic dome shaped shelters that do not interfere with urine and faeces collection. These protocols have been planned to cause the least pain, suffering or distress whilst adequately addressing the scientific question they have been designed to answer.