Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 16

Projects with a primary purpose of: Translational and applied research - Other Human Disorders

Project Titles and keywords

1. **Prevention of post-surgical adhesions**
    - Surgery, tissue adhesions, inflammation, tissue healing, experimental medicine

2. **Medicines Palatability Testing**
    - Medicine, Palatability, Patient Compliance

3. **Noninvasive Ultrasound for Therapy and Diagnosis**
    - Ultrasound, Cavitation, Therapy, Diagnosis, Drug Delivery

Project 1	**Prevention of post-surgical adhesions**	
Key Words (max. 5 words)	Surgery, tissue adhesions, inflammation, tissue healing, experimental medicine	
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)	Post-surgical adhesions remain one of the most common, unsolved complications following surgery. An adhesion is unwanted scar tissue binding organs and/or tissues together. This occurs as a repair process post-surgery and usually dissolves with time; however, it frequently becomes too excessive or persistent, causing complications. Excessive adhesions make staged or repeated heart surgeries difficult by increasing the risk of catastrophic bleeding by damaging the heart or large vessels. Adhesions can also cause occlusion of coronary artery bypass surgery. Following intra-abdominal and pelvic surgery, adhesions can cause bowel obstruction, female infertility, and pain. Adhesions may also limit future treatment options, such as minimally invasive surgery. The only effective therapy for post-surgical adhesions is corrective surgery, once it has developed. Therefore, prevention of post-surgical adhesions is of great value; however, no effective drug has been	

	developed for this purpose. Instead, an increasing number of medical devices, including artificial films and gels, have been commercialised. However, the therapeutic effects and availability of these products are so far unsatisfactory. Therefore, we aim to develop a more effective, more widely-applicable and more user-friendly anti-adhesion treatment.
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 results obtained will provide pre-clinical evidence to validate new therapies to prevent post-operative adhesions, enabling their further development forwards clinical application. Thus, this project has great potential to help patients who have risks to develop post-surgical adhesions and save the cost to treat such patients. In addition, this project will provide important new biomedical and scientific knowledge to understand the mechanism for adhesion formation and shed light on new biological insights, which will pave the way to future medical and scientific research. Therefore, this project potentially has a great impact on patients, doctors, scientists, the NHS and society, and there are also commercial opportunities associated with the innovative therapy for post-surgical adhesions.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use approximately 500 mice and 200 rats 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?	Animals are expected to recover uneventfully from anaesthesia and surgery. No mortality is predicted by the protocols, and animals are expected to experience discomfort and pain only during the immediate post operative period when it will be limited with pain-controlling drugs. Any animal suffering unexpected discomfort will be humanely killed. All animals will be humanely euthanized at the end of the protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The proposed project aims to develop a new treatment to prevent post-operative adhesions. In order to evaluate therapeutic effects of the treatment,

use non-animal alternatives	measurements of the degree of post-surgical adhesions after the treatment are needed. The process to form post-surgical adhesions is extremely complicated, involving multiple cell types (both locally resident and recruited) as well as many molecules and signalling pathways. Although we have made all possible efforts, it is impossible to represent such complex processes by using computer-based systems, lower organisms and embryo stages, cultured cells, tissue, and organs. Only living animals can be meaningful models for the purpose.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A wide literature search has confirmed that this project is original, and that there is no duplication with previous reports. For quantitative experiments, animal numbers needed are statistically set using power analysis. To assure reproducible outcome, which will maximises the information obtained from the minimum resource, experiments will be carefully designed and performed, including randomisation of treatment or control, allocation concealment, and blinded assessment of outcome, explicit inclusion and exclusion criteria. In addition, tissues from the same animal will be used to as many analyses as possible to minimise the number of animals required.
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 rats and mice, which are the most suitable for this basic/preclinical investigation; basic pathophysiology on post-surgical adhesions is sufficiently compatible between human and rodents. In the rodent models, we will be able to use genetically altered animals. The proposed project requires evaluation of post-surgical adhesions in clinically relevant settings. Adhesion-inducing operation is the only appropriate experimental model to represent such a clinical process. In fact all previous reports have used this model to produce post-surgical peritoneal or pericardial adhesions. The adhesion-inducing operations are recovery surgeries and are classified to be “moderate severity”, but the period during which the animals experience this is limited to the immediate postoperative period

	when pain will be controlled with pain controlling drugs. Once the animals recover from surgery, they usually behave similarly to healthy ones. Post operatively, animals will receive intensive care for several hours in special recovery cages, where their body temperature is regulated and with a supply of concentrated oxygen. Post-operative pain will be treated using analgesics. Infection will be prevented by using antibiotics and aseptic procedures in a specifically-regulated recovery surgery room. Dehydration or hypovolaemia will be prevented by administration of fluids and limited blood collection. In the unlikely event that animals do not recover uneventfully from surgery or subsequently develop unexpectedly severe adverse effects (for example, bowel obstruction) they will be removed from the study and humanely killed.

Project 2	**Medicines Palatability Testing**	
Key Words (max. 5 words)	Medicine, Palatability, Patient Compliance	
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
	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)	Aversive taste of oral, inhaled or intra-nasal medicines is a major factor in limiting the willingness of patients, especially children, to take medicine. The aims of the project are to: 1. Identify aversive taste in potential medicines as early as possible. 2. Build up information about effects of salt forms, formulations, masking agents, etc. on palatability. 3. Provide information that enables medicine development plans to be adjusted to address potential palatability problems. 4. Conduct further validation of non-mammalian and /or non-animal methods for detecting averse taste and refine current animal testing procedures. The safety profile of potential new medicines is not known early in the medicine discovery process, so palatability assessments cannot be tested in humans at this stage.	

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)?	We expect to be able to identify taste issues early in discovery of new medicines, and either: 1. Find alternative compounds without taste issues 2. Make modifications (e.g. to the formulation) to make the medicine more palatable 3. Identify agents which might mask the aversive taste In addition, the information we gather will be used to inform the possible development of non-mammalian and/ or non-animal alternatives to test for palatability issues. We have validated the model against a range of marketed medicines of varying palatability, and found that it matches closely with the human experience. Further data with novel potential medicines which have been given to humans continues to support this. We are, therefore, confident that we can predict accurately which potential medicines will be aversive to patients. The overall benefit will be more palatable medicines resulting in improved patient compliance, the possibly more rapid development of medicines, and increased clinical benefit to those with unmet medical need.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat – 750 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?	During the testing session (which lasts 30-45 min), each rat is presented with a series of water bottles containing different concentrations of test compounds in drinking water. The number of licks from each bottle is assessed automatically – adverse tasting solutions result in a reduced licking rate compared with that of water. Animals require 2-3 testing sessions, over 2-3 days, to become acclimatised to the procedure and produce reproducible results. To provide the rats with an incentive to drink when in the test rig drinking water is withheld for 21-23 hours

	before each testing session. We have monitored the animals' biology and behaviour extensively, and have found withholding water for this period produces only mild and transient effects e.g. <10% body weight loss. Body weights return to normal rapidly once the animals are allowed free access to water on completion of the test session. Recovery is carefully monitored by a veterinary surgeon and the licence holder. Once fully recovered these animals can be used in further studies while requiring less training than naive animals. All animals are humanely killed at the end of the final study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No fully validated non-animal alternative for the assessment of palatability currently exists. We are working with an academic group to see whether amoeba can be used to replace the current rat model. There has been some initial and promising success that will continue to be explored. We expect definitive answers on the usefulness of the amoeba model as a means to filter out compounds with obvious taste issues before they are tested in the rat, or as an alternative to the rat model during the course of this project licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice has been sought, and continues to be sought on a regular basis, to optimise study design and minimise animal usage. Re-use of animals familiar with the testing rig reduces the overall number of sessions required as well as the total number of animals. Our knowledge of chemical templates and/ or formulations with known taste issues would be used to decrease the number of any such compounds being tested.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Taste in rodents is very similar to humans, especially aversive reactions to bitterness. Non-mammalian species (e.g. zebra fish) are not considered to be suitable alternatives due to different mechanisms for

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	detecting taste. Several refinements to the rat model have been put in place over recent years. These include: • Reducing the duration over which water needs to be withheld • Rehydration in home cages – the more familiar environment (compared with the test cage) for the rats has beneficial effects (e.g. faster rehydration) • Refinements to statistical analyses • Fewer presentations of water bottles during the test, leading to shorter study duration • The cage in which the testing session is carried out has been modified to benefit the rats (e.g. improved flooring, more gradual exposure to the test bottles)

Project 3	**Noninvasive Ultrasound for Therapy and Diagnosis**	
Key Words (max. 5 words)	Ultrasound, Cavitation, Therapy, Diagnosis, Drug Delivery	
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)	The overarching aims of our research project are: 1. To understand the mechanisms of how ultrasound with or without acoustic particles interacts with biological tissue. Acoustic particles (e.g., microbubbles), can respond specifically to ultrasound in many ways, such as expanding and contracting or the release of drugs. 2. To design, create, optimise, and characterise novel technologies, acoustic particles, therapeutic agents, diagnostic agents, and procedures for the treatment and diagnosis of diseases.	
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 use of ultrasound and acoustic particles in the clinic has continued to increase over the last several decades both as diagnostic and therapeutic technologies. Ultrasound is expanding from its traditional use for imaging tissue structure towards high frame rate (>2 kHz) imaging, imaging tissue	

	function, and other capabilities. Meanwhile, therapeutic ultrasound is experiencing an even greater revolution of capabilities. Ultrasound has been can open capillaries to enhance drug delivery, dissolve clots to treat stroke, and a wide range of other therapeutic bioeffects, many of which we aim to understand and control here. A UK “Report of the Independent Advisory Group on Non-ionising Radiation” (Health Protection Agency, 2008) has identified concerns with these rapid changes and that there is (1) a knowledge gap of how ultrasound with or without acoustic particles can produce different effects and (2) how the effects can be controlled so that the desired objective can be produced while avoiding adverse effects in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	6,200 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 some animals, we will perform surgery. Pain will be controlled during these procedures by providing anaesthesia and pain medication. Good aseptic techniques will be used to minimise infections, which may occur in less than 1% of all procedures. Rare re-openirg of the wounds may be treated by re-closure under short- term general anaesthesia on one occasion only. In the unlikely event that animals show severe adverse symptoms, the animal will be killed immediately. Mice may suffer from hypoglycaemia from fasting. Every. effort will be made to reduce the duration of fasting while producing the sought diagnostic or therapeutic effect. Sugar may be fed during fasting, where evidence is provided that it will not effect the experimental results. Regardless, food will not be withheld longer than 16 hours. Animals inoculated with tumourigenic compounds are likely to develop tumours. We will not allow the tumour to reach a size that affects the overall health of the animal. As the tumour grows, potential adverse effects, such as tumour ulceration and a hindrance to

	ambulate, will be monitored closely. If signs of ill health are noticed an animal technician or expert will be notified and the animal will be monitored as appropriate. Palliative care may be provided as suitable. We will follow standard guidelines (LASA) when collecting blood samples. The amount of blood sampled will not exceed an amount that will effect the overall health of the animal. Following the collection of blood samples, the puncture site will be cleaned and monitored in a manner similar to what we experience at the hospital.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The ultrasound technology developed here aims to perform non-invasive diagnostic and therapeutic procedures on biological tissue. One of the main challenges with studying these phenomena and methods is that ultrasound interactions occur at the micron-, and nano-scale 3-dimensionally. Current 3D tissue culture models are unable to model complex and clinically relevant microenvironments. Also, ultrasound reflects strongly to material with a high acoustic impedance mismatch, such as glass and hard plastic materials, which are commonly used for tissue culture setups. These mismatches alter pressure waveforms in tissue culture setups that may never exist in vivo. Therefore, only a higher mammalian organism can be used to reproduce many of the important 3D tissue microenvironments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce the number of animals used in our experiments by using alternative methods: • Theory, computer simulations, and experiments on tissue-mimicking material will be extensively used prior to in vivo experiments to select, validate and optimise the biological model and the ultrasound parameters. • Long studies using the same mouse that do not significantly increase the severity of the protocol allow for overall reduced number of animals to be used.

	During the experimental design stage of the studies, we. will ensure that the data obtained throughout each stage will provide as clear of a conclusion as possible while using as low a number of animals as possible.
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 were chosen because they are the lowest animals on the evolutionary tree for which many suitable models of diseases (e.g., cancer) are available. Subcutaneous cancer tumours will be visualised by eye and measured by callipers. Tumour size will be monitored and the major discriminating factor to ensure the animals do not experience any discomfort. The sizes of tumours used under this licence are unlikely to affect the health of the animals. Haematological cancers, orthotopic tumours, tumours growing in transgenic animals, and other cancers not observable by the naked eye will be monitored by imaging when appropriate. Adverse effects. due to the administration of therapeutic or diagnostic agents will be monitored in accordance with NCRI guidelines for the Welfare and use of animals in cancer research. LASA Good Practice Guidelines on the Administration of Substances will also be followed. When we want to use ultrasound in a way that does not effect the biological tissue, ultrasound exposure conditions will be selected to remain within clinically defined limits. However, the purpose of many of our applications is to understand and control how ultrasound alters the biological tissue structure and function to optimise beneficial effects while minimising adverse effects. As a result, some of our investigations will use exposure conditions that will alter the tissue, The following principles will be followed in order to refine our exposure conditions: • Reduction of the region of ultrasound exposure by careful positioning of the ultrasound transducer(s) or by focussing ultrasound to a small target region.

	• Limiting exposure parameters that are not contributing to the advancement of our understanding.