Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 24

Project Titles and key words

- The neuropharmacology of learning and memory

  neuropharmacology; associative learning; memory; rat

- Evaluation of centrally-acting drugs

  Drugs CNS Efficacy Side-effects

- Optimising immunotherapy for autoimmune disease

  Autoimmune diabetes, autoimmune diseases, Immunotherapy

- Examining FMDV infectious processes in small animals

  FMD, Antibodies, vaccines, epitopes, capsids

- Antibodies for research and assays

  Antibody, Monoclonal, Neuromuscular, Diagnosis, Monitoring

- Molecular mechanisms of dendrite development

  Kinase, neuron, development, autism

- Processing of sensory information in the brain

  Brain, neurological disorders, neurons, information processing

- Functional Analysis of Maternal Factors in Zebrafish

  Embryonic patterning, maternal factors, Nodal signalling, RNA localization, non-coding RNAs

- The Production of Laboratory Animal Bioproduct

  Blood plasma serum tissue fluid

- Cattle vaccine strategies against bovine tuberculosis

  Bovine Tuberculosis, cattle, vaccination, DIVA, Biomarkers

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The neuropharmacology of learning and memory</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>neuropharmacology; associative learning; memory; rat</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<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></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>When there is a time gap between events, we are less able to make a connection between them in learning and later memory. Thus it is harder to keep track of things that could in fact be causally related, in order - for example - to know that even distant engine noise can predict a future hazard or to anticipate dinner based on the smell of raw ingredients. The ability successfully to bridge a time gap between events is known to deteriorate with age. This project will investigate the underpinning brain substrates of this important cognitive ability in relation to its psychological bases. One possibility is that the mental image of the first event fades before the association at issue can be made. An alternative possibility is that other intervening events interfere with the association between the first event and the outcome event. The differential role of time versus interference will be examined in experiments which systematically compare how learning is reduced by a time gap between the end of the first event and the start of the outcome event, relative to the reduction in learning produced by events of extended duration which do not terminate before the start of the outcome event (such that the overall time duration to be bridged is equivalent). In the first scenario the potential for competing events to interfere with conditioning is much greater. The proposed experiments will be conducted on rats using highly controlled presentation of events such as a distinctive noise followed by a mild footshock.</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

	Because the 'events' presented in such controlled experimental studies are somewhat artificial (e.g., a pure tone stimulus) we will also test the effects of the same interventions in the brain on the recognition of three-dimensional objects. In a complementary series of experiments we will present the objects with and without a time delay before familiarity is tested, with and without the presentation of intervening objects. The brain interventions will be precisely targeted in both cortical and sub-cortical areas implicated in these processes. The present project will advance on previous findings in that we will selectively interfere with chemical signalling within these pathways, both by depleting brain chemicals in specific pathways and by targeted drug delivery. Specifically, the aim of the present project is to systematically compare the role different chemical neurotransmitter pathways (e.g., DA, 5-HT) within the brain cortico-striatal-hippocampal circuitry across a series of behavioural tests suitable to model age-related decline in cognitive function. The specific objectives are to: 1. test the effects of DA and 5-HT depletion in subregions of nucleus accumbens and prefrontal cortex on trace conditioning and object recognition after a time delay; 2. clarify the role of time versus interference using delay conditioning controls and relative recency object recognition variants; 3. identify key DA and 5-HT receptors mediating neuromodulation of identified behavioural effects in brain regions of interest; 4. to clarify the underlying psychological mechanisms using comparison behavioural tests by way of positive control; 5. to clarify the underlying neurochemistry using comparison drug treatments by way of positive control. [507 words]
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 behavioural tests in use have validity as models of the cognitive changes seen in age-related decline. The original contribution of the present project will be to compare the modulatory effects of activation or inhibition of different neurotransmitter receptor families at different sites of action within the brain. [48 words]
What species and approximate numbers of animals do you expect to use	Rat: up to 2880 over 5 years.

over what period of time?	
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 most significant adverse effect is likely to be discomfort after surgical procedures. We will operate to the highest standards and carefully monitor the animals postoperatively. We should not exceed the moderate severity limits and will aim to keep below these limits at all times. The animals will be humanely killed at the end of the experiments. [57 words]
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Only in animals can we carry out these direct manipulations of neurotransmitter systems to test their role in conditioning over a trace interval. Computer simulations cannot substitute for experiments until we have sufficient data of the type this project will provide to successfully model the real nervous system. Our behavioural testing is intended to assess cognition and this requires the use of a relatively complex organism. We use rats because we are interested in the mammalian brain and the fundamentals of rats' learning and memory are not qualitatively different from those seen in human subjects. [95 words]
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are interested in relatively big effects to identify critical brain systems (rather than systems that play some minor role) and the proposed sample sizes (of 12 per group) are fully consistent with projected sample estimates based on known effect size (Cohen 1992). Moreover, this sample size will be sufficient to allow for exclusions based on verification of the intended neurotransmitter depletions or cannula placements (in the micro-injection studies). For each and every experiment, as part of good laboratory practice, we will write an experimental protocol to include details of the number of animals to be used and methods of data analysis. The results from powerful factorial designs will be suitable for analysis of variance. [115 words]
3. Refinement Explain the choice of species	We propose to use rats rather than mice or some

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.	other small mammal to make use of the huge body of evidence already collected on the rat (both behavioural and neuroanatomical). There are excellent stereotaxic atlases for rats and a wealth of behavioural studies that we make use of in selecting our experimental parameters. Rats are also a hardy species, well able to tolerate the water or food deprivation necessary to motivate responding. The aversively motivated fear conditioning procedures use mild footshock, just sufficient to produce reliable associative learning and within just two conditioning trials. Thus these procedures allow us to refine our microinjection procedures as the number of injections which can be administered without causing local damage at the point of infusion is limited. We will explore the viability of repeated micro-injections to test effects on the acquisition of trace conditioning in a 4 day variant of the appetitive procedure run with an increased number of trials per day. In order to ensure that high quality, reliable and valid data is extracted from the minimum number animals, the NC3Rs guidelines will be followed, also with respect to reporting the results – http://www.nc3rs.org.uk/page.asp?id=1357

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Evaluation of centrally-acting drugs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Drugs CNS Efficacy Side-effects</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Psychiatric and neurological conditions affect hundreds of millions of people worldwide and are an emotional and financial burden to patients, their friends and families and society. Drugs currently available to treat CNS disorders are hampered by limited efficacy and/or effectiveness, delayed onset of action and unacceptable side-effects including drug abuse liability. More efficacious and safer drugs to treat CNS disorders are urgently required. The main purpose of this project license is provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the efficacy, mode of action and side-effects of novel drugs and novel pharmacological targets for the treatment of CNS disorders. There is a real demand for these services from pharmaceutical and biotech companies due lack of appropriate expertise or laboratory facilities in-house and/or capacity issues. These specialised techniques may occasionally be employed to evaluate centrally-mediated side-effects of drugs to treat other medical conditions.</td></tr>
<tr><td>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)?</td><td>Experiments conducted under this licence will expedite the development of better drugs to treat CNS disorders, allowing faster access to safe and efficacious drug therapies for these serious medical conditions. Assessment of centrally-mediated side-effects of drugs to treat other medical conditions will also assist in their development and could reduce the time for them to reach the market. In addition, wherever possible, information will be disseminated into the scientific community. This will further knowledge about novel</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	molecular targets in the CNS and the efficacy and/or safety of new drugs to treat CNS disorders and other conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5000 rats and 2500 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?	The majority of studies to investigate the efficacy, mode of action or side-effects of centrally-acting drugs will involve simple dosing (acute or chronic) by an appropriate route with blood or tissue sampling for pharmacokinetic, biochemical, histological or neurochemical analysis and/or behavioural/physiological testing. Some drugs may already have been tested in vivo by the client (before being sent to us for evaluation in models that the clients do not have themselves) and would not be expected to produce any adverse effects. Occasionally, substances will be evaluated which may not have been tested in animals before and may produce unexpected toxic effects which could cause pain, suffering and lasting harm or in extreme cases death if humane end-points were not applied. Occasionally, drugs will be given centrally or peripherally by continuous infusion from osmotic minipumps or administered directly into the brain or a vein. These methods will involve anaesthesia and/or surgical procedures for subcutaneous implantation of minipumps, indwelling cannulae into the brain or intravenous catheters and it is possible that post-surgical complications may occasionally arise or the animals may experience post-operative pain. In some cases, the animal models employed may involve induction of specific pharmacological responses and/or involve training in specialised equipment which may produce transient discomfort/stress. For these reasons, the likely/expected level of severity of the license is moderate. At the end of procedures animals will be terminated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternatives to the models employed as they are used to assess the integrated behavioural and/or physiological/pharmacological responses of the whole animal to different treatments. All drugs to be evaluated will have been extensively characterised in vitro. However, as information obtained in cell-lines, cells and tissues relates to only part of the animal, it cannot replace ex vivo or in vivo tests. Such animal testing is a fundamental requirement for progressing novel

	agents into man and for dossier submission to the regulatory authorities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be minimised by only testing drugs in assays relevant to their pharmacological profile; measuring several parameters in the same animals wherever possible and continued use of animals (where animal welfare and experimental data will not be compromised). A fully qualified, highly experienced biostatistician will advise on experimental design and ensure that the minimum numbers of animals are used to produce meaningful statistical comparisons.
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.	Rats and mice will be used as details about their central nervous system have been well-documented and they are the lowest form of mammal that can provide meaningful data about man. Most studies will employ normal healthy animals (generally adult, on rare occasions as young as one month). Occasionally, genetically-altered animals may be used to model specific CNS disorders or provide proof of concept for novel targets for treatment of CNS disorders. Animals displaying adverse phenotypes will not be used. A variety of established, fully-validated animal models and assays will be employed. These have been widely used by the pharmaceutical industry to predict the effects of drugs in man. Animal models that produce unnecessary suffering will not be used. Substances will be given by the least severe route of administration. If substances have not been given to animals before, pilot studies will be performed. Simple acute screens will normally be conducted before chronic or complex assays. In general, the behavioural/physiological tests employed are well-tolerated. Exposure to these tests will be kept to the minimum necessary to obtain the data or for the animal to learn a given behaviour. If substances are given as part of the procedure to induce an animal model of a CNS disorder or a specific pharmacological response, doses will be carefully chosen and experiments designed so that any adverse effects and/or the duration of time that animals are exposed to the adverse effects are the minimum required to enable scientific objectives to be met. Surgical procedures will only be used if alternatives are not available. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Aseptic operating procedures, topical application of suitable antiseptics and plastic dressing will be used to reduce the possibility of infection. Post-operative analgesia will be used as advised by the NVS to

	reduce pain and suffering. All animals will receive the highest possible standard of post-operative care. The project is supported by a dedicated animal husbandry and technical support team. Studies will be conducted by staff highly-experienced in animal handling. Animals on study will be monitored closely and veterinary advice will be promptly sort should it be needed. Consideration will always be given to ways to minimise any welfare costs to the animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Optimising immunotherapy for autoimmune disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autoimmune diabetes, autoimmune diseases, Immunotherapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<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[6]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our overall goal is to optimise the use of molecules that modulate the function of immune cells for treating autoimmune diseases. Our current focus is the interleukin-2 (IL-2) pathway; the cytokine IL-2 is essential for regulatory Tcells' maintenance and for immune homeostasis.</td></tr>
<tr><td>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)?</td><td>The main benefit from this research programme is the discovery of information that can be used to improve the design of human immunotherapy trials for the autoimmune diseases type 1 diabetes and multiple sclerosis, both of which are increasing at a rapid rate in industrialised nations.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse.
25,000 mice will be bred of which about 75% will be part of various studies over the 5 year course of the licence.</td></tr>
<tr><td>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?</td><td>Some of the mice will develop diabetes – the main disease that we are aiming to cure. Due to genetic mutations in some of the knock-out mice we use there are certain expected phenotypes such as lymphomas within the thymus that we keep a careful look-out for. For the MS model that we will use we will be monitoring the small number of mice very carefully for the first signs of paralysis. We understand fairly precisely the rate at which this will occur and so know exactly when to monitor mice over the critical stages. At the end all mice will be killed by a schedule 1 method and/or carried over into the next project licence.</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Autoimmune disease involves many interacting cells that have developed and migrated within the autoimmune-prone environment and so the whole body must be analysed to understand the progression of disease over time. Similarly, whole animal models are essential for the testing of therapeutics because in addition to the disease process needing to occur *in vivo*, the PK/PD of the delivered drugs can only be appropriately monitored *in vivo* and no alternatives exist.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of mice bred, strains not being used continuously are frozen as embryos and breeding stopped. Group sizes for experiments are minimised in consultation with our statistics group so as to make sure that we are sufficiently powered at all times. Most *in vivo* experiments are done on groups of 3-6 mice and experiments repeated 2 or 3 times to be confident of results. Depending on the observed variability, group sizes may be adjusted but experimental protocols are not usually pursued if they require a group size of greater than 6 to achieve a 0.05 P value with the appropriate statistical test.
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 we maintain a number of strains that develop immune abnormalities or autoimmune disease, our long experience in closely monitoring such mice has allowed us to minimise any suffering and make full use of these valuable models leading to substantial insights into immune pathogenesis and immune therapies. Since many of the adverse phenotypes (eg. diabetes, thymoma, etc.) are age-dependent, we strive to utilise mice at a young age. Much of our work using mice is done *ex vivo*. Where *in vivo* experiments are performed they are largely mild and short term. Use of the mouse models where immuno-deficient mice are reconstituted with human immune cells facilitates the testing of species-specific therapeutics, whilst also replacing to some extent the need for work in non- human primates.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Examining FMDV infectious processes in small animals.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>FMD, Antibodies, vaccines, epitopes, capsids</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of the work covered in this project licence application is to identify the targets on the surface of FMDV that are recognised by protective antibodies of the host immune system. This will help us to select the most appropriate vaccine strains and to develop more broadly cross-protective vaccines.</td></tr>
<tr><td>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)?</td><td>The benefits of the work under this project licence will be an improved understanding of the antibody-mediated protection against this exotic virus in a small animal model. By looking at how well these viruses are recognised by antibodies from immune animals and how well each virus is able to induce a FMD protective immune response, we build up a picture of the contribution of each viral component to antibody mediated protection. We can then make accurate and more rapid predictions of which vaccines will offer protection against a new strain of FMDV. This assists the decision of whether and where to apply emergency vaccination in the face of a new FMD incursion; effectiveness of any vaccination programme is dependent on the speed of its implementation. This knowledge will also help to develop broader spectrum FMD vaccines that will improve the prospects for global FMD control and eradication.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Our plans are to use up to 336 guinea pigs over the five year life of the project; reviewed periodically through our internal Ethical Review Process.</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

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?	Some animals will be vaccinated to study their antibody responses to viruses with various changes introduced into their surface features and some animals will be inoculated with live FMDV to test whether or not the antibodies elicited by the vaccines provide protection. All the animals are closely monitored and their body temperatures are checked by a microchip to ensure their wellbeing. In guinea pigs, FMDV rarely causes signs of serious illness. They are examined at frequent intervals for health, appetite, weight loss, temperature and clinical scoring and if this occurs animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will use cell culture techniques where possible for virus isolation, stock production and titration. The newly constructed viruses will be evaluated in in-vitro tests to find out how well each one is recognised by antibodies from immune animals. This will give an indication of which immunogenic features of the virus could induce a protective immune response. Only confirmatory tests are done in vivo where justified by in vitro findings. It is difficult to do these studies in the large animal natural hosts because of the numbers of animals required. Therefore, these findings will be confirmed in vivo, in a guinea pig model, which has been used for studying FMDV infection for the last four decades.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experiments proposed benefit from knowledge of published studies in peer reviewed scientific journals and have incorporated advice on experimental design for analysis from a professional biostatistician in our institute. The number of animals proposed for the animal experiment is considered to be an acceptable balance between keeping the number minimum and fulfilling the requirement for obtaining statistically valid results.
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.	Guinea pigs are the most suitable small animal model for these studies as FMD in guinea pigs is similar to that in large animals such as cattle, and usually, the disease is rather mild. We consider that there is not a more refined way to conduct the in vivo studies described in this Project Licence.

Project Title (Max 50 characters)	Antibodies for research and assays		
Key words (Max 5 words)	Antibody, Monoclonal, Neuromuscular, Diagnosis, Monitoring		
Expected duration of the project (yrs)	5 years		
Purpose of the project (as in Article 5)	Basic research	Yes	
	Translational and applied research	Yes	
	Regulatory use and routine production		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals		No
Describe the objectives of the project (eg the scientific unknowns or scientific/clinical needs being addressed)?	To produce and characterise novel monoclonal antibodies for use in biochemical research. The protein targets against which the antibodies bind will be relevant to human diseases. New protein targets may be identified frequently as new discoveries are published in the scientific literature.		
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 monoclonal antibodies will be used in our own laboratories and in collaborating laboratories worldwide for basic research into human diseases, primarily neuromuscular disease, to improve diagnosis and to monitor potential therapies.		
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 200 mice and 30 rats over a period of 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?	Adverse effects may possibly be moderate and may be sores/ulceration at the site of injection or haemorrhage at the site of blood withdrawal. Animals showing pain, distress, and discomfort will be humanely killed (schedule 1). At the end of the protocol, animals will either be killed by a schedule 1 method or blood withdrawn by cardiac puncture under terminal anaesthesia.		
Application of the 3Rs			

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Antibody production without the use of animals has not been shown to produce the range of antibody specificities and affinities required in this project. If a suitable non-animal technique becomes available, it will be used to replace the live animal work as soon as practicable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A minimum number of animals will be used for each antigen. Initially, we typically immunise 3 mice, though many other researchers use more than this. The 3 mice often show different immune responses. Occasionally only one mouse will produce antibodies of the required kind. By experience we have found the initial immunisation of 3 mice with each antigen is a good compromise in that animal numbers are kept low, but there is a good chance of getting at least one animal with the required response within the time frame of the initial series of immunisations. If a satisfactory mAb is already available commercially or on free distribution, we would not make new mAbs to duplicate it.
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	Many different species-specific secondary antibodies are now available, but mouse is still the species of choice for monoclonal antibodies used in many applications. Freund's complete adjuvant (FCA) is considered the gold standard for adjuvants by many immunologists and the general immuostimulatory properties of FCA have not been surpassed by any other adjuvant. Reaction to FCA will be minimised by using a newer formulation, minimising injection site volume and using the subcutaneous route. Wellbeing, pain and distress of animals will be monitored regularly.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Molecular mechanisms of dendrite development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Kinase, neuron, development, autism</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our main goal is to discover novel mechanisms that play important roles in brain and specifically dendrite development. Kinases are enzymes that have critical functions in all cells. Role of several different kinases in neuronal development is not well understood. Learning more about kinases and defining the molecular pathways that they regulate could be useful in designing therapeutics for neurodevelopmental diseases.</td></tr>
<tr><td>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)?</td><td>For example several neurodevelopmental diseases have genetic causes. Identification of the genetic cause is becoming more widespread now with the advance of gene sequencing and completion of human genome project. Kinases regulate all cellular process. Using the information generated in this project drug targets might be identified which can be used for therapeutic interventions of neurodevelopmental disorders.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We anticipate using 6650 mice and 500 rats in 5 years. The majority of mice will be genetically altered in various kinase signalling genes.</td></tr>
<tr><td>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?</td><td>In vast majority of cases we will be killing the animals, mostly under terminal anaesthesia to remove tissue for histological or physiological examinations. We will also use standard killing procedures to kill animals without anaesthesia. Animals will be killed at the end. Breeding genetically altered animals are non-invasive. We have carried out biochemical and cell culture experiments which, in combination with information</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

	obtained from public access databases helped us identify candidate genes that are required for the formation and maintenance of the nervous system. We used this information to obtain or start generating transgenic animals in which the expression of selected genes is modified. Our main experimental approach involves histological and microscopic analysis of post-mortem tissues and physiological analysis of organs isolated from transgenic animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our project's goal is to discover mechanisms that play a role in the formation of connections between brain cells with each other. Brain circuitries that we are planning to work on to understand the molecular basis of their formation cannot be produced elsewhere. Non-animal models cannot replace testing hypothesis, that may be generated by the non-animal work, in animals. Nevertheless, much of the work in this project is to be done using ex-vivo material. Prior work from other groups and our in vitro work supports this project. Before embarking on any animal experiments, we will collect as much evidence as possible to determine whether a candidate genetic or environmental manipulation has a reasonable chance of success and providing information within *in vivo* systems. Evidence will be collected from our own experiences and previous results as well as by surveying the mammalian and other literature. In addition, we will use non-regulated procedures to collect expression data from fixed non-GM mammalian tissues and functional/expression data from genetically and/or environmentally manipulated *in vitro* mammalian cell lines and/or early chick and fish embryos.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use statistical tools to ensure that we only use as much animals as needed to produce meaningful results. Most of our work will be conducted ex-vivo. When possible we will keep the transgenic lines as homozygotes. Based on previous experience we expect that we will need no more than 10-14 animals per condition to obtain meaningful results. Severity: Mild/ non-recovery. We will make maximum use of each animal used in this protocol/ We aim to collect organs from different organs to freeze and store for sharing with appropriate scientists. In this way we will maximize the information obtained from using animals. For protocol 4 we will use wild type mice or rats that come from central supply or commercial sources, not non-transgenic littermates from Protocol 1, or GA animals received directly from other projects that will not be maintained on breeding protocol.

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 and rats are the species of choice because the brain circuitries that we plan to study are similar to humans as they are vertebrates and mammals. In addition, electrophysiological techniques from brain slices of rodents are well-established and rodents are widely used for mapping brain circuitry and identifying molecular pathways that regulate their formation. There is a large selection of transgenic mice that allows us to knock down genes of interest in subpopulation of cells and visualize these cells. We will ensure that the mice and rats are regularly monitored. If there is any signs of pain, distress and lasting harm that exceeds our severity limit we will kill the animals. In all procedures will be done in accordance with the local and national guidelines to minimize pain, suffering, distress and lasting harm. We will seel local guidance from NVS/NAWCO to confirm the competency. Most of the work we propose are standard which we have previous experience in the lab and in the Insitute. We will carefully monitor each mouse strain as well as rats to see idthe information we have obtained are accurate and that animals don't exceed their set limit for pain, suffering and lasting harm.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Processing of sensory information in the brain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, neurological disorders, neurons, information processing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Considered in its most reduced form, information processing requires transformation of input into output patterns. Understanding this fundamental process is therefore central to our goal of understanding brain function. Such input-output processing has been extensively studied in single neurons. Extending this to neuronal circuits requires an understanding of the intricate interplay of not only cellular physiology but also knowing the identity and precise connectivity of the elements of the network. In the mammalian brain, due to its interconnectedness, sheer scale and numbers, such understanding has been largely elusive.

Thus, in this project, the objective is to contribute to the understanding of how neural circuits shape the flow of sensory information.</td></tr>
<tr><td>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)?</td><td>While understanding functional connectivity of neural networks is a key question in its own right, a growing number of cognitive disorders are linked to alterations in neuronal connectivity. This includes diseases as socially and economically devastating as autism spectrum disorders or schizophrenia that together are estimated to affect more than 1 in 100 people. If a comprehensive analysis of information processing in a small mammalian neural network can be achieved, this might be a key ingredient to enable more mechanistic understanding of such diseases as well.</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

What species and approximate numbers of animals do you expect to use over what period of time?	We will exclusively use mice and few rats. We will purpose breed 18,000 mice and 800 rats over a five year research program.
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?	Adverse effects are expected to be generally mild and only rarely moderate – genetic or pharmacological modifications will generally be only applied to a tiny region or very small number of cells in the brain; surgical procedures are well established and generally minimally invasive. A social and enriched environment will be provided wherever possible to minimise social stress. At the end of the experiment animals will be sacrificed and analysed ex vivo.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Information processing in the brain in health and disease is one of the last unchartered territories where our understanding is severely limited. Thus, as computer simulations need validated input to generate meaningful output, we cannot yet replace the direct observation of nature by simulations. As we aim to contribute to the understanding of the human brain on disease and the computations underlying behaviour we have to focus on a species that is both phylogenetically close to humans and genetically tractable. Thus, we have to focus on rodents, and especially mice that allow targeted genetic manipulations to create models of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the past we have established highly quantitative physiological and behavioural assays. This, together with our strong statistical background will ensure that we can determine and use the minimal number of animals that will generate meaningful, reproducible, valid results. As we have in the past, we will use extensive computational approaches to focus our experimental efforts and thus further reduce animal numbers. Finally, wherever possible we will use within animal comparisons to assess treatment consequences which will allow us to use paired and much more powerful statistical tools and thus further reduce animal need.
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	As we aim to contribute to the understanding of the human brain on disease and the computations underlying behaviour we have to focus on a species that is both phylogenetically close to humans and genetically tractable. Thus, we focus on rodents, and especially mice that allow targeted genetic manipulations to create models of disease.

(harms) to the animals.	To reduce animal stress and significantly increase animal welfare, we have worked hard to establish a set of natural behavioural paradigms where mice are group housed in enriched environments and left unperturbed by human interference. This not only ensures highest quality (reproducible) data but also minimises animal stress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Functional Analysis of Maternal Factors in Zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Embryonic patterning, maternal factors, Nodal signalling, RNA localization, non-coding RNAs</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</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[14]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of the proposed work is to understand the molecular mechanisms underlying non-coding functions of RNAs in the genome and axis formation in vertebrate embryos. We are studying control of embryonic patterning by maternal RNAs as an experimental paradigm to understand how non-coding RNAs and other maternal factors regulate key steps in early development.</td></tr>
<tr><td>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)?</td><td>This work is of significance to understanding of early embryonic development and non-coding RNAs function in the context of human congenital birth defects.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The zebrafish, Danio rerio, is the species that will be used in the majority of experiments.
Zebrafish: 14,000 fish over 5 years</td></tr>
<tr><td>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?</td><td>All procedures used are of “mild” severity.
Expected adverse effects:
Anaesthetising fish can occasionally produce bleeding from the gills, which is short lived. These fish may recover but they will be observed carefully for signs of breathing difficulty or other distress. Fish that struggle to recover in fresh water will be killed by schedule 1.

Where the technique also involves brief removal</td></tr>
</table>

[13] Delete Yes or No as appropriate.
[14] At least one additional purpose must be selected with this option.

	from the water for detailed observation, the fish will be kept damp by being wrapped in a moist sponge having first established adequate anaesthetic depth has been achieved; this is a standard technique and in our experience is most unlikely to cause detectable harm. For fin-clipping, an appropriate anaesthetic will be chosen which is suitable for the procedure and in line with best practice. Anaesthesia will be carefully monitored throughout the brief procedure. Observation will continue until the fish recover consciousness and are able to swim normally. Adult fish heal very well naturally, and the amputated fin will regenerate within a few weeks to its normal size and shape. Infection following fin biopsy is rare, and has not been reported by experienced workers. Operated fish will be monitored carefully at regular intervals upon return to the aquarium system. If infection occurs, the fish will be killed by a Schedule 1 procedure. *If any doubt exists as to the severity of the adverse effects, then the named persons will be contacted and their advice will be followed.* In rare cases fish may carry dominant mutations that have deleterious effects on development and survival. Animals displaying aberrant phenotypes (such as tumourigenesis) will be identified by regular screening and immediately killed by a schedule 1 method. Spontaneously induced recessive alleles that are deleterious may occasionally arise in inbred fish. These will also be killed by a Schedule 1 method as soon as detected. If newly obtained mutations appear wild-type during embryonic stages, small numbers of animals (10 homozygotes) will be raised to test for adult phenotypes. Such animals will be checked every day for abnormalities initially and after two weeks every workday. If significant abnormalities are found such fish will be killed by a schedule 1 method, or by decapitation under terminal anaesthesia if this is required for further analysis. The animals will be killed by schedule 1 at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The overall aim of the proposed project is to understand the function of RNAs in embryonic development, and their implications for congenital birth defects. This requires study of complex tissue interactions in the ovary and embryo. Thus, it is not

	feasible to recreate this process accurately in cell culture. Animals are therefore absolutely necessary for this work. The analysis is focused on zebrafish transgenic lines or mutant lines that we will obtain or generate. We are studying the functions of an RNA with non-coding activity, and that of its binding proteins *in vivo*, and both components require animals. The zebrafish embryo has special advantages for co-localization/imaging studies, genetics and proteomics. Our choice of animal therefore reflects the 3Rs since fish are the simplest vertebrate model system in which these studies can be performed, and our experiments are on embryos that have a lower level of awareness in comparison to adults.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We estimate that we will use 14,000 adult zebrafish throughout the duration of the project, and the majority of these are generally only used for matings. These numbers are determined by stock keeping requirements, and a minimal number is required for each line to ensure maintenance or for generation of the required transgenic line. All unwanted lines and lines not in active use will typically be maintained as frozen sperm samples. Maximally 2500 of these will be used to generate 5 transgenic lines per year, the rest are required to maintain existing stocks. We will use maximally 200 embryos for gene and protein expression studies, and 200 adult females over the course of the study to understand processes and patterning events that occur in the adult ovary.
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.	All the experimental manipulations described in this proposal require easy access to embryos, and fish are a good model for the manipulations we have proposed in comparison to mammalian embryos. Thus, the work helps to address the 3Rs by using alternatives to mammalian models. Experiments will be carried out on embryos immediately after fertilization, and since the animals are under 5 days post fertilization, they do not fall under ASPA protection and are not as aware as adult animals or mammals. All embryos used for experimental purposes will be humanely killed at the end of the experiment, and will not be raised beyond 5 days post fertilization. Adult animals that have reached the end of the breeding life (~about two and a half years of age) will be humanely killed by an overdose of anaesthetic.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The Production of Laboratory Animal Bioproduct</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Blood plasma serum tissue fluid</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 Years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td>
<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>Yes</td><td></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[16]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project will offer a centralised, expert service for the provision of animal derived bio products for researchers with no facilities, capacity or expertise to perform this part of the work themselves. The supply of these products from such a service will benefit biomedical science as we can offer continuity of supply, an efficient and expert service, high level of animal welfare, ethical consideration and traceable, high quality products. The products are collected either from animals under an anaesthetic from which they will not recover or from conscious animals (donors) that have samples taken as required or as part of a scheduled collection. Samples are taken using refined techniques and within defined limits.</td></tr>
<tr><td>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)?</td><td>The products are used for the development of new medicines in man or animals and for the calibration and validation of machines or systems used to support research. They may also be used to support other methods in research as an alternative to live animals. The use of this type of centralised service provides the benefit that multiple products can be supplied from individual animals and provided to clients who do not have suitable facilities, expertise or capacity to keep the animals and collect the products themselves. A further benefit of a centralised service is that a high level of technical expertise is developed by the personnel who do the sampling.
Simultaneously harvesting as wide a range of bioproducts (blood products, body fluids and tissues) as possible from the smallest number of donors helps reduce the total number of donors</td></tr>
</table>

[15] Delete Yes or No as appropriate.
[16] At least one additional purpose must be selected with this option.

	needed by scientists who would otherwise need to use their own donors for the individual items they need**.**
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use the spare animals from breeding operations or we will use animals which are unfit for use in other studies because they may have a physical imperfection or they may perhaps be too old or of the wrong gender for use as a live animal. Over a 1 year period, the following numbers of donors are likely to be used in this project: Animals sampled under general anaesthetic from which they will not recover: Dog 50 Rabbit 100 Ferret 10 Rat 10 Mouse 10 Guinea Pig 10 Conscious donors used for sampling Dog 40 Rabbit 10 Ferret 10 Rat 10 Mouse 10 Guinea Pig 10
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?	When possible, the animals are sampled while asleep under general anaesthetic and so they do not experience any adverse effects. When the sampling is complete, these animals are humanely killed by an overdose of the anaesthetic and so they do not wake up at the end of the process. If it is required that that the blood products or tissues are free from anaesthetics, in this project we can take samples from conscious donors in much the same way as when a human gives a blood sample. This involves minimal stress and the animals suffer minimal if any adverse effects from the sampling.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	When synthetic alternatives are not validated as suitable, live animals must be used to derive the bioproducts. There are certain studies that must be performed in laboratory animals, therefore it is necessary to use bioproducts derived from these same species in order to complete the range of tests, some of which are legally required.
2. Reduction	The use of blood products, tissues and organs that

Explain how you will assure the use of minimum numbers of animals	are obtained from animals that are not suitable for use in research or surplus to requirement reduces the numbers of live animals required for experimental studies. Blood products are necessary for the calibration of test systems and some bioassays. Using good quality blood components will improve the significance of test results in studies involving animals and therefore lead to improved scientific knowledge and a reduction in the overall number of animals required. As multiple samples can be obtained from a small number of live donors this reduces the need to individually euthanise animals for the purpose of taking each sample. Where possible an individual animal will be used for more than one purpose, for example, following a procedure for the collection of blood from an animal under anaesthetic from which it is not allowed to recover, tissues and organs may be harvested or the cadaver used for educational purposes.
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 choice of donor species is driven by market demand which is dependent on the scientific needs of research scientists. Under this project, we will only supply tissues from species for which we have suitable housing. All the species that we propose to use have well-established roles in preclinical research. All personnel involved in the blood sampling procedures are trained and are supervised or competent to perform the procedure. We consider our techniques to be the most ethical and refined methods of obtaining samples and they have been developed so as to cause minimal distress and pain to the animal. Blood sampling from an animal which will not wake up is conducted under a general anaesthetic and we have refined the technique so that we will cause the minimum amount of discomfort and distress to the animal when we anaesthetise it.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cattle vaccine strategies against bovine tuberculosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bovine Tuberculosis, cattle, vaccination, DIVA, Biomarkers</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[17]</td><td>
<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[18]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>

Objective 1: To develop cattle TB vaccines with better protective efficacy than BCG alone, and/or vaccines that do not compromise tuberculin skin test specificity.
Objective 2: To study immune responses in vaccinated and/or infected animals to identify correlates of protection and disease severity.
Objective 3: To develop and validate antigens that allow the differential diagnosis of infected and vaccinated cattle (DIVA) in experimentally vaccinated and/or M. bovis infected animals.
Objective 4: To evaluate the performance of diagnostic tests and antigens in the field using naturally infected and uninfected cattle.

</td></tr>
<tr><td>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)?</td><td>

Evaluation and development of cattle TB vaccines to identify those strategies that can be progressed further to field trials and eventual licensing.
Development of improved ante-mortem immunodiagnostic tests including so-called DIVA tests that can be used alongside vaccination strategies to prioritise those tests that can be further evaluated in large field trials and eventually OIE licensed.
Generation of scientific knowledge and Intellectual Property Rights.
Benefits to other susceptible domestic animal species (e.g. deer, South American Camelids,

</td></tr>
</table>

[17] Delete Yes or No as appropriate.
[18] At least one additional purpose must be selected with this option.

	goats, or companion animals (e.g. cats) that could be vaccinated with the same vaccines that will be effective in cattle.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle (Bos taurus), 2000
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?	Expected levels are mild (protocols 2 and 3) to moderate (protocol 1). Adverse effects would be due to vaccination, mainly local reactivity at injection sites; effects of infection which, although not intended to, could in extremely rare cases lead to systemic symptoms of bovine tuberculosis; other adverse effects due to experimentation such as cannulation at site of surgery. No adverse effects are expected from other sampling such as blood collection. Animals will be either discharged from the license (majority), or killed by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For TB vaccine development as well as the development of diagnostic reagents, no non-animal alternatives are available, and use of target species, cattle is unavoidable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical assessment of sample size will be guiding every experiments and number of animals used per group. This analysis will be based on previously published data, with professional statistical advice to be sought on a case by case basis.
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.	Cattle are the target species of vaccination and associated diagnosis and therefore the appropriate species to be used to achieve our stated objectives. The methods we will apply are standard technology used worldwide, some of which we have refined to reduced animal welfare costs to the animals. Defined humane endpoints will be used to minimise the harm caused by any of the procedures used.