Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 14

Projects with a primary purpose of: Regulatory Purposes

Project Titles and keywords

1. **Testing and Development of Vaccines/Therapeutics**
    - Influenza-Vaccines-Therapeutics-Efficacy

2. **Therapeutic approaches to anticholinesterases**
    - Organophosphorus poisoning; therapeutics; regulatory submission

3. **Efficacy pharmacology assessment - CNS**
    - Pharmacology, CNS, Allergy, Inflammation and Nociception

4. **Safety and Quality Control Testing**
    - Pyrogen Abnormal Toxicity Quality Control

5. **Regulatory Testing Using Embryonating Hen's Eggs**
    - Disinfectant, Efficacy, Extraneous, Agents

6. **Safety Pharmacology**
    - Potential new medicine-Safety, Pharmacology, Cardiovascular, Central Nervous System

7. **Surgical alteration of animals for supply**
    - surgery, animal, supply

8. **Regulatory and Investigative Toxicology**
    - Medicine, safety, toxicity, toxicology

9. **Batch potency and safety testing of Foot and Mouth Disease (FMD) Vaccine**
    - Potency Safety FMD vaccine

10. **Efficacy and safety of feed additives/ingredients**
    - Feed, efficacy, safety, farm animals

11. **Transgenic Mouse model for poliomyelitis**
    - Transgenic mouse, poliomyelitis, polio vaccines

12. **Early safety assessments and follow-up investigatory studies**
    - Safety, pharmacology, drug development, physiology

13. **Production of Medical Diagnostic Reagents**
    - Blood, agar plates, vaccine production

14. **Safety Evaluation of Substances Administered to Man**
    - Regulatory, Toxicity, Safety

15. **Acute Toxicology –Chemicals**
    - Acute, toxicology, chemicals

16. **Imaging Agents - Biodistribution For QC testing of Ceretec**
    - QC, Batch release

17. **Production of antibodies, antisera and blood products**
    - Antibodies, antisera, blood, antigen

18. **Production of Blood Products for Scientific Use**
    - Blood, horse, sheep

19. **Viral Vaccines (Batch Release)**
    - Vaccine quality control, vaccine safety

20. **Efficacy Pharmacology Assessments: Circulatory, Gastrointestinal and Endocrine**
    - Pharmacology, Circulatory, Gastrointestinal, Endocrine

21. **Metabolism and Pharmacokinetic Studies**
    - Regulatory, Metabolism, Pharmacokinetic, Animal

22. **Coccidia control methods in poultry and game birds**
    - Coccidia, Parasites, Control

23. **Reproduction Toxicology Studies**
    - Toxicology, safety, reproduction

24. **Measuring the Strength of Immunological Medicines**
    - Immunological, medicines, vaccines

25. **Assessment of Bioaccumulation in Fish**
    - Bioaccumulation, Regulatory, Safety, Chemical, Pharmaceutical

26. **Standards in Virology**
    - Vaccine, Virus, Disease

27. **Drug evaluation in pre-clinical oncology models**
    - Oncology, Cancer, Efficacy, Combinations, Pre-clinical

28. Regulatory ecotoxicology

- Ecotoxicology, Regulatory, Safety, Chemical, Pharmaceutical

29. RODENT TOXICITY, TUMORIGENICITY AND SAFETY STUDIES

- Regulatory, Rodent, Safety, Toxicity, Tumorigenicity

30. Antibody Production for Research, Diagnosis and Therapy

- Antibodies, sheep, goats, hens, donkeys

31. Toxicity in Macaques by Inhalation Administration

- Pharmaceutical, Regulatory, Primate, Inhalation

<table>
<tr><td>Project 1</td><td colspan="2">Testing and Development of Vaccines/Therapeutics</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Influenza-Vaccines-Therapeutics-Efficacy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>YES</td><td>Basic research</td></tr>
<tr><td>YES</td><td>Translational and applied research</td></tr>
<tr><td>YES</td><td>Regulatory use and routine production</td></tr>
<tr><td>NO</td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td>NO</td><td>Preservation of species</td></tr>
<tr><td>NO</td><td>Higher education or training</td></tr>
<tr><td>NO</td><td>Forensic enquiries</td></tr>
<tr><td>NO</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">This project aims to:

• Utilise the established ferret model for study of new and novel strains of influenza viruses, to understand the viral pathogenesis and the efficacy of influenza vaccines and/or novel therapeutics.
• Determine the mechanisms of viral replication, pathogenesis and transmission.
• Efficacy testing of influenza vaccines and therapeutics in order to develop treatments.
• Regulatory testing of licenced influenza vaccines intended for infant and adult populations.
• Further develop immunological assays to study the ferret responses to influenza challenge and vaccine/anti-viral therapies.</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</td><td colspan="2">This project will allow for the use of established animal models and their further development which will enable the conduct of studies into viral pathogenesis, transmission and efficacy of vaccines and antivirals that have most clinical relevance.</td></tr>
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project)?	The data produced under this licence will help select vaccines/antivirals that are likely to be effective against seasonal and potentially highly pathogenic influenza viruses that haver the potential to cause both epidemic and pandemic outbreaks. In addition it will plug knowledge gaps by the establishment of immunological assays to study the ferret responses to influenza and vaccine/antiviral therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year length of the licence, up to 2500 ferrets will be used.
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?	Potential adverse effects to virus challenge and anaesthetics have been identified.. Any adverse effects associated with the anaesthesia will be monitored and appropriate steps taken to minimise hypothermia and dehydration. Adverse effects associated with the viral challenge will be identified by detailed behavioural monitoring (nasal discharge, sneezing, and lethargy loss of appetite). Together with this behavioural monitoring, objective data (animal weight and temperature) will be collected and analysed. Adverse effects associated with the administration of substances will be minimised by using highly trained staff competent in the delivery of substance via various routes, by using the smallest of volumes and rate of administration of substances. In order to minimise the severity any significant changes in the above criteria will result in increased monitoring frequency, or the euthanasia of any animal. For example: the loss of 20% body weight of any animal will be the trigger to euthanise any animal by a Schedule 1 method or by terminal exsanguination under full anaesthesia. To assist in the prompt recognition and subsequent intervention, critical periods will have been identified and monitoring frequency increased to every six hours as a minimum. Humane clinical endpoints have been clearly defined therefore, unnecessary suffering is avoided. At the end of all studies, animals will be euthanised by a Schedule 1 method or by terminal

	exsanguination under full anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex interactions between the influenza virus and the host are difficult to model without the use of a living animal where the target cells, innate defence mechanisms of the respiratory tract and the innate and acquired responses of the immune system are as fully functional as they would be in man. Only in the animal model can we currently demonstrate efficacy in terms of alleviation of clinical signs and systemic pathology. Whilst limited trials can be conducted in human volunteers there are ethical and safety reasons that preclude the use of virulent strains and certain routes of administration.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals per group will be used to satisfy the power requirements of the study. The power and statistical value of the study is affected by the variability of the measured parameter. Statistical advice is available and this advice will be used to minimise animal usage. Studies will be conducted in a step-wise manner so that the number of animals used will be minimised if the treatment shows no likelihood of efficacy; for example, if a new vaccine does not elicit an appropriate immune response, then it would not progress to a challenge efficacy study. Robust scientific quality control of the test materials and methods will ensure that studies are carried out successfully first time, minimising the need to repeat studies and subsequently reduce the number of animals 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.	The work carried out under this licence will involve the use of ferrets as they possess numerous advantages over other animal models, making them the most suitable animal model for the range of studies proposed within this licence. As in humans, influenza illness in ferrets is acute and usually lasts 3 – 5 days. While several other animal models have been utilised to study influenza, unlike ferrets the pathogenesis does not resemble that in humans (nasal discharge, sneezing). Aspects of this licence rely on the ability to observe clinical signs in the infected animal, for example anti-viral testing

	upon symptom onset. In order to provide animals with maximum social interaction and environmental enrichment we will aim to group house, where possible, animals within the high containment facility throughout the duration of the study. Regulatory testing of live attenuated influenza virus (LAIV) involves relatively small numbers of animals per test and multiple test samples (same strain) can be run using the one control group which will subsequently reduce the overall number of animals used. The humane endpoint for all studies is a combination of detailed behavioural monitoring (nasal discharge, sneezing, lethargy and loss of appetite and objective data collection (animal weight and temperature). As this programme of work progresses, it is intended that data collected will inform the refinement of subsequent studies.i.e. Periodic review of humane endpoint 20% weight loss criteria to determine if this can be further refined. For studies where regular blood samples are required standard guidelines for delivery and removal of substances via various routes will be followed as outlined in 'First report of the BVA/ FRAME/ RSPCA/ UFAW joint working group on refinement '(Lab Animals (2007) 41, 321-328).

Project 2	**Therapeutic approaches to anticholinesterases**	
Key Words (max. 5 words)	Organophosphorus poisoning; therapeutics; regulatory submission	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	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 assess pre-treatments and/or treatments against poisons that act by inhibiting acetylcholinesterase. Objective 1. To assess, *in vivo*, the efficacy and/or tolerability of novel drugs and/or combinations of drugs for protective pretreatment and/or first aid therapies against anticholinesterase poisoning. Objective 2. To assess, *in vivo*, the efficacy and/or tolerability of drugs (both novel and established) and/or combinations of drugs in the follow-up medical management of anticholinesterase poisoning. Objective 3. To produce data regarding the efficacy of novel drugs/combinations of drugs for the treatment of anticholinesterase poisoning for submission to relevant regulatory authorities.	
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)?	More effective therapeutic strategies for anticholinesterase poisoning need to be developed. Unlike the development of many other drugs, there is no clinical population in which to test such drugs in a controlled way, so information gained from animal studies is critical for the development and licensure of these drugs for human use.	

What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 400-500 guinea-pigs per year through the duration of the project. In the latter 2-3 years of the project, it is possible we may transfer some studies to an alternative rodent species. These could use 250-300 mice or rats per year, with a concomitant reduction in guinea-pig usage.
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 anticholinesterases are extremely toxic, and poisoning can produce a range of effects from salivation, through muscular weakness to respiratory distress and in extreme cases death. The aim of the research is that animals receiving effective therapeutic drugs at the optimum doses and dose intervals will be protected from the most severe effects of poisoning. Some animals will display severe signs of anticholinesterase poisoning. Humane end-points will be used wherever possible and these are being refined and developed continuously. Some animals will receive only therapy drugs, to ensure that they do not themselves produce adverse effects. Some animals will undergo surgical procedures to enable more sophisticated monitoring of the effects the anticholinesterase and therapy. Some animals will have blood sampling lines implanted surgically to avoid the requirement for repeated sampling through the skin. At the end of each study, all animals will be humanely killed. This will allow further examination of some organs and tissues, and samples may be taken for microscopic examination if appropriate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Anticholinesterases affect a range of organs and organ systems, including the cardiovascular, respiratory, hepatic, renal, skeletomuscular, immune and nervous systems, including special senses (vision and hearing). Whole body systems are therefore required to study the toxic chemicals and the ability of pretreatments and therapies to protect against them. Unlike most drugs, it is not ethically possible to conduct full Phase III clinical trials in humans for new medical countermeasures using these types of toxic agents. It is therefore critical to be able to extrapolate

	from dose-efficacy in animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Many of the new therapeutic drugs will have previously been tested in *in vitro* systems. Only the most promising drugs will be tested in animals. Advice from statisticians on the experimental designs used will ensure that robust results can be obtained using the minimum numbers of animals. Data from control experiments will be shared wherever possible and LD_{50} determinations of toxic agent in the absence of any therapy will only be determined if no suitable data exist already.
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 have been widely used in previous work, and a large body of information exists against which new results can be compared. Generally, they are the best species from which to predict the response in higher animals and humans. Sometimes, rats may be used, due to biochemical differences between the animal species and humans. Genetically-modified mice are being developed that express “human-type” enzymes and suppress certain mouse enzymes that are particularly relevant to the study of these agents. In all studies, we aim to maximise the information obtained to the animal at minimal welfare cost. This means that some animals will be monitored using sophisticated telemetry equipment; however this requires a surgical procedure under general anaesthetic. For some studies, it is more appropriate to use a combination of observations by trained personnel together with basic temperature monitoring using equipment not requiring the animal to be operated upon. Humane end-points will be invoked where possible and these are being refined and developed continuously. Where blood sampling is required to measure levels of therapy drugs or challenge agent, it is generally less stressful for the animal to have a sampling cannula implanted under general anaesthetic than to take repeated blood samples through the skin.

Project 3	**Efficacy pharmacology assessment - CNS**	
Key Words (max. 5 words)	Pharmacology, CNS, Allergy, Inflammation and Nociception	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	No	Basic research
	No	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
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The studies to be conducted under this licence are not intended to satisfy any particular regulatory guidelines. However regulatory authorities are increasingly requesting to see good quality proof of concept data when considering approval of new medicines. The Community code relating to medicinal products for human use (Directive 2001/83/EC) requires understanding of the intended and unintended pharmacological effects of novel medicines.	
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)?	In the case of medicinal products, achievement of the objectives of this licence enables the identification of candidates to progress into clinical testing and to pre-marketing authorisation. The information will also be used by scientists and responsible clinicians for selection of both starting and limit doses for clinical studies and to identify parameters which should be clinically evaluated. Without these studies progression of new treatments to early human studies and to patients/marketing could not occur.	
What species and approximate numbers of animals do you expect to use	Over the 5 year life of this Project Licence, it is estimated that 9,900 mice, 9,800 rats and 200	

over what period of time?	guinea-pigs will be used.
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?	Humane endpoints (documented within the licence) will be applied to animals used under the protocols specified in this licence. The severity limits specified are considered to be the minimum commensurate with achieving study objectives. In the majority of studies conducted under this licence, the severity limit is moderate and most animals will not experience more than mild signs. For a minority of models performed under this licence surgical implantation of catheters or recording devices is required which raises the initially level of severity to a moderate. In such cases animals are anticipated to show minimal or no adverse effects resulting from surgery following recovery. Lead optimisation / efficacy studies are conducted early on in the drug development process, typically prior to regulatory safety studies. However, it is expected that preliminary data will be available, in particular pharmacokinetic data which will enable dose levels to be selected which have the potential to show efficacy, with minimal adverse effects. Tests within this licence are designed to be closely related to human disease conditions, the majority of these are expected to result in mild or moderate adverse effects. In a number of protocols e.g. those relating to arthritis, epilepsy and multiple sclerosis, some animals may experience up to severe adverse effects. For such studies the animals are closely monitored and humane endpoints modified to ensure that the welfare of the animal or the scientific purpose of the study is not compromised in any way. In addition, the environment within the home cage is modified for the well-being of the animals, for example by the addition of soaked food pellets and additional bedding material. Although these models can cause adverse effects, the test agents used in these models are anticipate to alleviate the clinical signs associated with the disease state and as a result the majority of animals display a notably reduced severity. The majority of animals will be humanely killed at the end of a study.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Several in vitro techniques such as receptor binding, in silico modelling and functional high throughput assays are used during early drug development and provide an early indication of the potential therapeutic properties of the drug. However, to fully assess the pharmacodynamic effects (effects of a drug on the body) of a new drug in vivo testing is necessary. Only in a fully operational circulating system can the drug's distribution, metabolism and excretion, which may alter or intensify the efficacy or adverse effects of the new medicine, be fully understood. For these reasons animal models remain essential in the development of new medicines. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.
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.	Rodent models are adequate to complete these types of studies and the use of species at a higher level of neurophysiological sensitivity has been avoided Where scientifically possible animals will be assessed under anaesthetic. However due to the effect of anaesthetic on certain body systems and the relatively short duration of anaesthesia, this is not often possible. For example, prophylactic or therapeutic time-course effects cannot be assessed in anaesthetised animals, nor can the desire to self-administer a drug. Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare

Project 4	**Safety and Quality Control Testing**	
Key Words (max. 5 words)	Pyrogen Abnormal Toxicity Quality Control	
Expected duration of the project (yrs)	5	
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
		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)	Human and veterinary drug products must be effective and safe so their production is carefully monitored to make sure it complies with the Good Manufacturing Practice. Quality control tests must be performed on samples from each batch to comply with national and EU regulations.	
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 test procedures detailed in this license form an essential part of the data required to monitor the safe development, production and release of medicinal products for safe use by consumers. The ability to test these products ensures that they have been produced safely and to appropriate standards. These results provide satisfaction to the relevant Marketing Authorisation that the tested products are safe to use by the end consumer. The prevention of the tests listed would result in the release of these products being delayed or denied. Depending on the exact nature of the medicinal product this could lead to serious health implications for the intended patients. As the nature of the this work is quality control on each batch produced any prevention of testing would have an immediate impact.	

What species and approximate numbers of animals do you expect to use over what period of time?	Pyrogen Test: Rabbit – 4000 over 5 years Abnormal Toxicity: Mice – 500; Guinea Pig – 50 over 5 years Absence of Toxicity: Mice – 500; Guinea Pig – 50 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 aim of the Pyrogen test is to identify substances which may cause a pyrogenic response. Adverse effects may include: a rise in core temperature of 1.2°C during a study (experience over several years has shown this would occur in less than 1% of the animals but positively prevents some potentially harmful product from going into the market place); damage to the ear veins may occur with the repeated injections; occasionally there may be unexpected response to the test substance. The aim of Abnormal Toxicity is to test products that are considered to be safe and therefore adverse effects are uncommon as they are not expected except for some clinical response to specific drugs (e.g. antibiotics). Adverse effects will occur when the test product causes a reaction in the animal and maybe observed if any animal appears hunched, to have lost weight, lethargic or in a poor condition; or displays a loss of appetite or reduced water intake. In the case of mice, animals may appear in obvious distress and may vocalise. Guinea pigs may appear unusually quiet.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The methods listed are specifically required by legislation and/or regulatory authorities to confirm safety of the product, leaving little opportunity for non-animal methods. It is not possible at present to reproduce in-vitro the complex composite whole body response produced in vivo and the availability of validated alternatives for regulatory use is somewhat limited. However where ever possible, non-animal alternatives are used. When available, clients are required to demonstrate that non-animal test are not appropriate. In addition certain screens can usefully be utilised to rule out some products before in-vivo testing; however these have limitations and can only be used for some compounds. Bacterial Endotoxin Testing has been offered as a service for many years and clients have been actively encouraged to use this format of testing rather than the Rabbit Pyrogen Test. In some cases BET has not been suitable and

	Monocyte Activation Testing is currently being validated in order to provide non-animal tests for these products. Testing will not be carried out for 3rd countries if a non-animal alternative is accepted by the legislation of the EU.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The methods listed are closely defined and must be carried out to a strict protocol for compliance with the requirements of the various regulatory authorities and there is therefore little opportunity to reduce animal numbers used in any specific procedure. However tests done to meet the requirements of non-EU regulators will only be performed if they use the same or fewer animals and the end points are no more severe than the EU equivalent.
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 animals used for the various procedures have been established over many years and found to be appropriate to monitor the safety of the product by the consumer. In most cases the species required are specified absolutely by the method as required by legislation and/or regulatory authorities.

Project 5	**Regulatory Testing Using Embryonating Hen's Eggs**
Key Words	Disinfectant, Efficacy, Extraneous, Agents
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
	(b) translational or applied research with one of the following aims:
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

To test batches of veterinary vaccines for the presence of contaminating organisms.

To provide evidence of the effectiveness of disinfectants against Newcastle Disease Virus and Avian Influenza Virus.

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)?

Assurance to the poultry industry that batches of veterinary vaccines are not going to cause disease rather than preventing it. To provide assurance that, in the event of a disease outbreak in poultry, effective disinfectants are available to help in control.

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

30,000 chicken eggs

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

For the 7,500 eggs used for vaccine batch testing, less than 5% are expected to die and these will be before the age at which the Home Office considers the embryos to be self-aware. For the 22,500 eggs used for disinfectant testing, a percentage of eggs will die. But again, the vast majority of deaths will occur before the age at which the Home Office considers the embryos to be self-aware. All surviving embryos are euthanised by a schedule 1 method by the time they reach 18 days of age.

Application of the 3Rs

Replacement

The tests used are based on Government legislation which specifies the test system to be used.

Reduction

The government regulations also specify the numbers of eggs to be used.

Refinement

The tests used are based on Government legislation which specifies the test system to be used.

Experienced technicians will monitor the eggs daily to ensure any embryos showing signs of distress are euthanised at the earliest opportunity consistent with the aims of the work.

Project 6	**Safety Pharmacology**
Key Words	Potential new medicine-Safety, Pharmacology, Cardiovascular, Central Nervous System
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Understanding how safe a potential new medicine is before it is given to humans is an essential part of medicine development. Although some information on safety can be obtained without using animals, some tests must be carried out using animals to better understand how these medicines might affect the intact human body.

The aims of the project are:

1. To identify the effects of medicines with potential impact on safety of patients or human volunteers taking part in clinical trials. Investigate and understand the mechanisms of potential safety issues and avoid them during development of potential new medicines.
2. To improve and refine our tests to provide more relevant information and, where possible, to be more relevant to humans whilst minimising the use and impact on animals.

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 will identify pharmacological safety issues in the discovery or early in the development of new medicines and either: • Find alternative compounds without these safety issues • Make modification to the chemistry or method of delivery to make the medicine safer • Understand safety liabilities associated with specific targets or chemical structures and avoid these in future research. In addition, the

information we gather will be used to help further develop our computer and non-animal based safety assessment models.

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

Mouse - 300 over 5 years Rat - 2650 over 5 years Guinea-pig - 200 over 5 years Rabbit - 1200 over 5 years Pig - 350 over 5 years Dog - 500 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 levels of severity? What will happen to the animals at the end?

Of animals in this licence 36% will be exposed to non-recovery procedures where appropriate anaesthetic will be used to minimise any adverse effects and achieve the scientific goals of the study. All other animals on this licence will experience moderate adverse effects at worse. Animals implanted with recording devices, are used (41% of animals) to investigate the cardiovascular effects of potential medicines, will undergo recovery surgery. These animals will have appropriate care including analgesia, antibiotics, special dietary support and intensive monitoring to aid recovery. All recovery surgical procedures will be carried out under aseptic conditions and recovery is expected to be uncomplicated, where complications do arise (e.g. swelling, redness or discharge from operative site) treatment will be started on veterinary advice and if no improvement is seen, or the condition worsens, the animals will be euthanased. Our previous experience shows rodents having recording probes implanted display mild loss of hind limb function, reduced weight bearing and limping in approximately 20% of cases. Such animals are closely observed and if no improvement is seen or the condition worsens animals will be euthanased. The remaining 23% of animals undergo preliminary evaluation of physiological function, or used on non-invasive imaging studies, and are unlikely to induce more than mild adverse effects. We endeavour to test only pharmacological doses of medicines under evaluation however we occasionally see unexpected adverse effects. Such effects may include reduced food consumption, abnormal behaviour (e.g. subdued responsiveness, vocalisation or hunched position), abnormal breathing and when observed advice will be sought from the veterinary surgeon (NVS) on appropriate remedial action. If the adverse effects are marked or remedial action does not improve the condition of the animals they will be euthanased. Animals used in evaluation of novel medicines, particularly those with implanted recording devices, may be retained under the care of the NVS for subsequent re-use in this licence. Following completion of an initial, or any subsequent procedure, the NVS will determine if the animal is fit to be retained and all such animals will undergo a health check prior to any subsequent re-use. The overall lifetime cost to the animal will be the main component in making the determination of fitness. At the end of procedures animals not retained for re-use will be euthanased.

Application of the 3Rs

Replacement

All work using animals will be preceded by studies using human cell lines, human stem cells, computer based evaluations and isolated tissues from animals. Data from

these studies are used to selected appropriate compounds and doses for evaluation in animals.

The core systems of the body, the circulation, central nervous and respiratory systems, are all maintained by complex interdependent control mechanisms which at present are impossible to recreate in a “test-tube”. While we investigate specific aspects of these systems in simple models to determine the effects of prospective medicines on the integrated control functions we must use intact animals where such function can interact with each other. There is a large body of work which shows that the models used have a good degree of translation to the effects seen in man.

Reduction

Extensive use of computer and cell based evaluations limits the number of potential medicines being evaluated in whole animals. Furthermore, all of our study designs are reviewed by statisticians to ensure that we use the optimal design and minimum number of animals to achieve our objectives.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Following on from our non-animal testing approaches potential medicines will routinely be evaluated in isolated tissues prior to evaluation in rodents. Only those compounds of interest, or with specific liabilities that can only be evaluated in dogs or pigs, will progress into these species.

The telemetry technology described provides robust data of the highest quality, in animals that remain undisturbed during evaluation and are thus exposed to minimal stress. Investigational work requiring many complex recording systems will be carried out in non-recovery animals under terminal anaesthesia. Evaluation over repeated administrations of potential medicines may use imaging technology to combine functional and structural readouts.

Animals will be routinely group housed to allow socialisation unless the recording systems being used requires single housing, the duration of which will be kept to a minimum. The number and volumes of administrations and blood samples will be minimised while ensuring scientific validity and minimising discomfort. Where possible, animals will be acclimatised to procedures. Environmental enrichment will be provided in home and recording cages or pens.

Project 7	**Surgical alteration of animals for supply**
Key Words	surgery, animal, supply
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

This is a service licence to produce surgically prepared rats, mice, hamsters, guinea pigs and rabbits and supply them to research establishments in the UK.

They will either be used in regulated scientific procedures under a project licence or used in non-regulated procedures, in which case a project license would not be required, such as for vasectomised animals.

These surgically altered animals will be used for a range of objectives, such as:

Animals implanted with a vascular catheter will be used in studies with any of the purposes indicated above where repeated or continuous intravascular injections would otherwise have to be made or where repeated blood sampling would be required. Implanting these animals with a catheter reduces the need for repeat needle sticks and restraint which improves the welfare of the animals themselves as well as the quality of the scientific outcome of the study.

Animals that have their ovaries removed can be used in basic research into the physiology of and applied research into the treatment for women who are post-menopausal or who have had their ovaries removed.

Vasectomised animals can be used in the production of genetically altered animals. Genetically altered animals are widely used for research into genetic conditions of people and animals and their treatments.

To protect the environment, new chemicals need to be tested to make sure that they do not have a sex-hormone (such as oestrogen) effect. This type of safety test needs to be performed on animals that have their ovaries removed or have been castrated so they are not producing their own sex hormones.

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 benefits of surgically altering the animals in this project are multiple and change depending on what they will be used for. These animals will support the research into human and animals diseases including genetic diseases. They are also likely to be used in the development and safety testing of medicines which can benefit humans and animals. Some of these animals will enable protection of the environment through safety testing of chemicals. Placing catheters in the blood vessels of animals provides a benefit to the animals themselves (less restraint and needle sticks) as well as enables the benefit of the outcomes of the research in which they are used.

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

The surgery will be performed in rodents and rabbits. The numbers used will depend on the demand for surgically altered animals for use on the final project licences they are destined for. It is expected that a maximum of 8100 animals will be surgically altered during the lifespan of this project licence.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The potential level of severity is moderate as all the procedures will involve surgery. The best anaesthesia methods will be used and good pain relief will always be provided. Expected adverse effects would be pain and discomfort post-operatively, loss of catheter patency (i.e. unable to take blood sample due to a clot forming over time) or post- operative effects from the anaesthetic e.g. slight loss of body weight. At the end of the procedures on this project licence the animals will be transferred to other relevant project licences for use in scientific studies or used in non-regulated procedures, in the case of vasectomised animals.

Application of the 3Rs

Replacement

Only orders for Surgically altered animals will be accepted of which the project licence holder is satisfied that justification exists for the use of these animals and that no non-animal alternatives are suitable.

Reduction

Customers will be asked to justify the numbers of animals they are requesting before work is accepted. Our company Culture of Care is committed to the 3Rs, as are our customers. This service is focused on ethical review of the work we undertake, which our customers appreciate as this too is their focus. Surgically preparing unnecessary spare animals is not only un-ethical but time-consuming and financially costly for customer and supplier. As such there are multi-factorial reasons to use only the number of animals required to achieve the scientific objective.

The animals scientific study use at the customer's facility is subject to Ethical Review and application of the 3Rs also under their project licence requirements.

Refinement

Surgical preparation only begins when appropriate checks have been made to ensure that the necessary authorities are in place to allow the recipient to receive surgically altered animals and to perform studies using them. The scientific background for each individual use is therefore specific to each customer, their research requirement, justification and their Project Licence authority.

All surgery will be performed by verified competent surgeons, using aseptic technique in line with best anaesthetic and analgesia (pain-relief) practices. All animals will be closely monitored by experienced technicians before, during and after surgery to ensure any refinements in their husbandry, surgical procedures or post-operative maintenance can be carried out. Records will be kept and reviewed to audit the surgery procedures to allow for continual refinement.

The surgeons will participate in continuing professional development and sharing of best surgical practices with peers.

The activities are overseen by the project licence holder and both the NACWO and NVS participate to provide advice and expertise on animal health and welfare.

Project 8	**Regulatory and Investigative Toxicology**
Key Words	Medicine, safety, toxicity, toxicology
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Understanding how safe a potential new medicine is before it is given to humans is an essential part of medicine development. Although some information on safety can be obtained without using animals, some tests must be carried out using animals to better understand how these medicines might affect the human body. The objectives of this project are as follows:

- Identify the right potential medicines for development which are safe to give to people and are most likely to be able to treat the target illness.
- Identify any possible safety concerns and understand how these might arise and whether they could cause harm to patients or human volunteers in clinical trials.

Where possible, improve and refine our tests using animals to provide more relevant information to humans whilst minimising the use and impact on animals.

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)?

Achievement of the objectives will support development of safe, new medicines to improve health and quality of life of patients by generating high quality, regulatory acceptable data and will help to remove unsuitable candidates from the development pipeline at an early stage, thus minimising the use of animals and resources. The benefits gained by studies performed depends on the study purpose and type and include: Making decisions on whether potential new medicines are suitable for development as early as possible in the process to avoid wasting animals and

money. We use the information generated during early studies to help to understand what we need to measure on future studies. To help us to decide the doses and endpoints to measure on early human studies to minimise the risk to human volunteers. To allow regulatory authorities to decide whether to allow the potential new medicine to be given to humans.

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

Over the 5 year period of the licence we expect to use approximately: 5500 rats 2000 mice 575 dogs 200 hamsters 200 rabbits 575 pigs

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Evaluation of safety to assess potential risk to humans requires the use high doses of a potential new medicine which can cause some adverse effects in animals. Adverse effects in animals are usually of mild or moderate severity. The most common effects will be loss of body weight or reduced weight gain, reduction in the amount of food the animals are eating and clinical signs such as reduced activity, postural changes, changes in faeces and in some species, vomiting. No animals will intentionally experience severe adverse effects but because early studies may be the first time that a potential new medicine is given to animals, effects may occasionally be more severe than expected. Animals are monitored closely and animals which show signs toward the limit of moderate severity are humanely killed. Most safety studies require examination of blood and tissues from animals to see whether the potential new medicine has caused any damage to organs or tissues, so the majority of animals are humanely killed at the end of a study and subjected to post mortem examination. Samples of tissues are then examined microscopically. On some early studies animals are not required to be killed and provided they have not shown adverse effects they may be used again in a subsequent study.

Application of the 3Rs

Replacement

Whilst alternatives to in vivo animal models are being developed and are used where possible, there are currently no reliable models available for broad, primary toxicity screening and none that are acceptable to drug regulatory authorities, it is therefore necessary to screen for toxicity in animal models

Reduction

For safety studies, guidelines require the number of groups, and animals per group to, be adequate to clearly demonstrate the presence or absence of an effect of the test substance. We have a track record of designing studies that provide us with the information we need to make decisions on the safety of our test substances (leading to continuing, or stopping, development).

For preliminary studies, small groups are acceptable because of the endpoints used give a clear answer. Where group sizes are sufficient data from definitive toxicity

studies are analysed statistically. Statistical input is sought, where necessary, to strengthen the overall scientific quality and relevance of the studies to be performed, with sample size calculations performed for specific studies to determine the group size. Group sizes in dog and pig studies are usually insufficient for valid statistical analysis. However, because toxicity is the result of changes in multiple parameters, assessment is made by examination of data from each animal and by correlation of in-life and post mortem findings within an individual.

In order to minimise animal use, we will consider using animals on more than one study when this can be justified on welfare and scientific grounds.

Refinement

Regulatory guidelines state that toxicology studies in support of administration to man should be conducted in one rodent and one non-rodent species. Generally the rat is the rodent species of choice unless it is known to be an inappropriate model for man for the compound. The non-rodent species will be that likely to give the most satisfactory, reliable and regulatory acceptable results.

The pig will be used as the preferred non-rodent species in this licence, unless it is shown to be unsuitable based on scientific information available, when the dog will be used. Where evaluation of all information indicates that both the pig and dog are a suitable non rodent species, the pig will be chosen.

Project 9	**Batch potency and safety testing of Foot and Mouth Disease (FMD) Vaccine**
Key Words	Potency Safety FMD vaccine
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

To ensure that each batch of FMD vaccine produced is safe and effective for use in field.

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 availability of an effective FMD vaccine, is the cornerstone of many national governments strategies to prevent of FMD outbreaks with their associated animal welfare problems and social, economic and environmental costs.

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

1550 cattle, 1970 pigs and 260 sheep over the 5 years

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

There are two procedures on this licence, both use a sample of FMD vaccine which if it passes both of these tests will go on to be commercially used on animals in various parts of the world. Both protocols are mild, the safety test uses a double dose of the vaccine to assess if there any adverse effect (temperature rise and swelling at the injection site). If there is an adverse effect on the health and welfare of the animal , the animal will be humanely euthanased. The potency test uses the prescribed dose and has one or two blood samples taken, there is no expectation of adverse effects.

Application of the 3Rs

Replacement

There is no in-vitro system as the complete biological system required for these tests.

Reduction

The numbers are dictated by the European pharmacopeia or if not , by national government standards

Refinement

For the safety test the European pharmacopeia specifies the most sensitive category of animal to measure any adverse effects of the vaccine of each target species of the vaccine (this usually means the youngest age indicated). The safety test involves the injection of a double dose of the commercial formulation of the vaccine with temperature monitoring

The original method of assessing vaccine potency was a challenge test with live FMD virus.

The European Directive 81/852/EEC indicates that methods other than the challenge method stated in the pharmacopoeia for determining the potency of FMD vaccine may be used provided that a statistical evaluation has established a satisfactory correlation between the two methods. The correlation between the level of circulating neutralising antibody in animals vaccinated with the batch under test and the level of protection against infection has now been well established and all the major FMD Institutes and vaccine producers now use the in vitro assay of neutralising antibody level directly as a measurement of vaccine potency. This means the test has reduced to one injection with commercial formulation of the vaccine and then a single blood sample. The European Pharmacopeia specifies cattle, or any other species for which immunogenicity has been shown. There is now an increasing requirement of national governments for the tests to be carried out in at least one the target species of the vaccine is intended for.

Project 10	**Efficacy and safety of feed additives.**	
Key Words (max. 5 words)	Feed, efficacy, safety, farm animals	
Expected duration of the project (yrs)	5	
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
		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 project aim is to generate data by conducting *in vivo* efficacy and safety studies in the target animal species to be used: - to develop and market feed additives, - in dossiers submitted for the registration of feed additives, - to determine the nutritive value and appropriate diet inclusion rates of novel feedstuffs. To protect human health, animal health and the environment, feed additives have to undergo an efficacy and safety assessment. European Parliament and Council Regulation (EC) No 1831/2003 states only additives that have been through an authorisation procedure may be marketed. EFSA is responsible for conducting the evaluation of the data submitted requesting authorisations. Authorisations are granted for specific animal species, specific age of animal, specific conditions of use and for ten year periods. The publication EFSA Technical Guidance (2011) -	

	Tolerance and efficacy studies in target animals, gives guidance on how to conduct and report studies concerning *in vivo* efficacy and safety (tolerance) studies.
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)?	Project benefits are to develop feed additives and ingredients that have the potential to improve animal performance health and welfare, are safe, reduce waste and have a positive impact on the environment.
What species and approximate numbers of animals do you expect to use over what period of time?	The project covers all farmed species; pigs, chickens, turkeys, ducks, sheep, goats and cattle. The anticipated number of animals will be a maximum of 32,000 in five years. Typical numbers vary from 20 dairy cows to 450 broiler chickens per study.
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?	Procedures undertaken enable the collection of biological material e.g. blood, faeces and urine, to determine the effect of the diet on metabolism or digestibility. Procedures are unlikely to cause adverse effects in excess of mild severity. The protocol allows for moderate severity of up to 20% weight loss in animals (except poultry) to enable assessment of tolerance feeding levels. Control measures and determination of endpoints are clearly defined. In most cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that the moderate severity limits are not exceeded. Where adverse effects are observed we will intervene to ensure that severity limits are not exceeded. Where at all possible, animals will be returned to farm following veterinary assessment that they are fully recovered from the procedure and pose no risk to human health, animal health or the environment. Where this cannot occur (an unregistered product for example), animals will be killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Feeding studies need to be undertaken in the target animals because food producing species have different digestive systems e.g. ruminant and mono-gastric, and the nutritive value of an ingredient is different between species. EFSA states that efficacy and safety studies must be undertaken in the individual target animal to enable authorisation for the use of feed additive products in a specific species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where there is a European guidance document detailing the animal requirements, we will comply with these. Where there is no guidance document, we will take the advice of a statistician when constructing our study design. The study design is determined to ensure significant differences between animal performance or biological measurement are achieved, if the animal responds differently to a treatment, with the minimum number of animals.
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.	Feeding studies need to be undertaken in the target animals because food producing species have different digestive systems e.g. ruminant and mono-gastric, and the nutritive value of an ingredient is different between species. EFSA states that efficacy and safety studies must be undertaken in the individual target animal to enable authorisation for the use of feed additive products in a specific species. Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent inspection by veterinary surgeons. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed and the harms to be experienced by the animals. Recommendations on refinement of procedures are a frequent outcome of this process. Animal health and welfare are paramount and enrichment is given in the pens, this will be bedding, where possible, and 'toys' e.g. chains for pigs.

Project 11	**Transgenic Mouse model for poliomyelitis**	
Key Words (max. 5 words)	Transgenic mouse, poliomyelitis, polio vaccines	
Expected duration of the project (yrs)	5	
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
		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)	Live-attenuated polio vaccines (oral polio vaccine or OPV) have been the principle tool used in the polio eradication programme of the World Health Organisation so far. They imitate natural infection of the gut, immunising recipients with a high degree of safety and efficacy. They are manufactured mainly in Europe where they are subject to EU regulations, which require testing of a proportion of batches in animals by Official Medicines Control Laboratories (OMCLs) designated by the licensing authorities. The test in transgenic mice carrying the human receptor for poliovirus has largely replaced the test in non-human primates that were previously the only adequate models for testing vaccine safety. This transgenic mouse assay has been established by WHO through collaborative studies and is detailed in WHO and European Pharmacopoeia regulatory documents, which specify the procedure, numbers of animals and the particular line of mice to be used. They have been designed to minimise animal usage and suffering while producing statistically reliable	

	results. The procedure involves the inoculation of a small volume of vaccine and animals are then followed for a period of two weeks and scored daily for signs of paralysis. Two doses of vaccine are tested along with a reference preparation; the vaccine passes if it is statistically no more virulent than the reference. It is possible to produce batches of vaccine that fail the test and in rare instances the vaccine can cause poliomyelitis itself. Thus testing of every batch is necessary and currently there is no alternative to the use of animals although the developmental aspects of the work to be undertaken aim to explore alternatives based on molecular biology which will be validated against the mouse test. The test will also be used to qualify new attenuated strains to be used for vaccine production as part of the global polio eradication final stages. Finally there is rising interest in the use of inactivated polio vaccines (IPV) and other non-infectious vaccines including the use of new strains and antiviral compounds whose effectiveness needs to be evaluated. The mouse model provides a way of assessing protective efficacy and will be increasingly important for this purpose.
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 most immediate benefit of the proposed programme will be that vaccines will continue to be scientifically assessed with quality assured methods which is essential to the continued success of the polio eradication programme which depends on the supply of vaccines of high quality and safety. The use of this licence will also significantly reduce the use of non-human primates for testing vaccines both in the UK (if still required) and in Europe. In addition, new vaccines and antivirals against poliovirus will be evaluated early in their development giving some confidence in their likely protective efficacy before their use in human subjects. Finally, results obtained with this test can be compared to those from other possible assays, particularly laboratory tests not requiring the use of animals, giving greater

	confidence in their reliability, applicability and validity for the batch release of vaccines. Therefore, the flow-through benefits of this work will have ramifications at the national, European and global level.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, including genetically altered. Up to 30,000 mice are expected to be used in 5 years (20,000 bred at our Establishment).
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?	Experience has shown that the genetically modified lines to be produced under this licence have no adverse effects on the mice and that the genetically modified traits under investigation have no adverse effects. There is a surgical procedure performed to expose the tissues around the specific area of the mouse's spine to ensure accurate inoculation. This is done under anaesthesia and analgesia is routinely provided to all animals before the procedure: the surgical wound is repaired after inoculation. The expected level of severity of transgenic mouse test ranges from mild to moderate depending on the protocol. Typical clinical signs of polio start with weakness of the limbs, which may develop into partial or full paralysis. There is no evidence of pain associated with the progression of polio symptoms which are rather characterised by loss of neural sensation. Animals with any clinical signs of polio will be monitored more frequently and when showing signs sufficient to allow conclusive assignment to a treatment group will be killed by Schedule 1 method. There is a small risk of infection developing at the closed incision site and/or animals showing injection trauma after inoculation. Regular monitoring ensures affected animals are treated quickly and/or euthanized should their condition not improve. Any animals that are unable to readily feed or drink as a result of their condition will be killed immediately. Veterinary advice will be sought in all other cases of variation from normality.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The transgenic mouse neurovirulence test is well defined in regulatory documents. It is intended to replace the test in non-human primates and has been validated for this purpose which has helped significantly reducing the need for non-human primates for OPV testing. The inherent complexity of the vertebrate immune system precludes the use of non-animal models for the assessment of vaccine-induced immunity. However, molecular tests have been developed to measure the proportion of a mutation associated with a virulent phenotype at certain positions in the viral genome. The test is included in regulatory requirements but has still not been shown to provide the same level of information. In addition, the advent of deep sequencing makes it theoretically possible to scan the entire genome and establish variability at certain positions with effect in virulence, or to demonstrate their value as markers of manufacturing consistency and differences between producers. This approach will be pursued in parallel with the in vivo based tests; if validated appropriately, in part against them, it could ultimately lead to the disappearance of the need for animal testing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The design of the regulatory test was based on intensive statistical discussion and input to define the minimum number of animals required to give valid results. They are detailed in official regulatory documents. With respect to the developmental work, statistical input is sought as part of the process of experimental design to ensure that the minimum numbers of animals are used. Additionally, there is a full control of the training process for this technique, which means that operators can be selected based on genuine experience of proficiency in Regulated Procedures, and can be monitored closely and constantly by experienced operators to determine genuine competence. This will help reducing the number of invalid tests and the unnecessary

	repetition of procedures. The number of animals used under the breeding protocol will be minimised through effective breeding colony management. Experiments will use an equal number of both sexes to minimise the numbers used under the 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.	The only animal alternative to the mouse model is the non-human primate: work to develop the mouse model has allowed the replacement of primate testing. The particular strain of mouse most commonly used is required as all validation work was conducted using this strain. This programme will also demonstrate the utility of mouse strains other than the strain used in the regulatory test. Animals are examined daily in the course of the studies and clear humane endpoints have been defined. For some studies it will be possible to refine the endpoint further depending upon the scientific information that is required. For infection protocols the period of monitoring clinical signs will be extended beyond normal working hours as required to make sure any harms to the animals are minimised, through effective application of the defined endpoint.

Project 12	**Early safety assessments and follow-up investigatory studies**	
Key Words (max. 5 words)	Safety, pharmacology, drug development, physiology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
	x	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 investigate the safety of potential new medicines by assessing their effects on vital body organ function (heart, lungs, central nervous system) in small laboratory animals. The studies will be performed on behalf of Sponsors (pharmaceutical/biotech companies/academic groups).	
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 data generated will provide an early indication of the potential side effects of new medicines in development. The studies may also provide useful information on the biological mechanism of the side effects. This information helps the Sponsor eliminate unsafe medicines and make necessary changes to the drug molecules to make them safer for animals, in future animal testing, and humans in clinical trials.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (120), Rats (1090), Guinea Pigs (610) over 5 years. A typical study will use relatively small numbers of animals (<40).	

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 adverse effects are likely to be caused by side effects of the medicines being tested. In approx. 60% of studies, the animals are expected to experience mild adverse effects such as slight weight loss and subdued behaviour. In 40% of studies, moderate adverse effects may occur such as more marked weight loss, reduced eating and changes in appearance (e.g. ruffled fur in rodents) or behaviour (e.g. reduced activity). Humane end-points are applied as necessary to minimise the suffering of the animals. Adverse effects may also occur as a result of the dosing procedure. Animals will be dosed by routes similar to those used in humans, e.g. by mouth (orally) or by injection and these require short-term restraint or confinement. Some animals may experience short-term discomfort following surgery to implant telemetry devices to measure the function of the heart or to implant intravenous catheters for longer duration injections. In separate studies, some animals may experience adverse effects due to lack of social contact since assessment of lung function requires temporary single housing. Transient discomfort may occur during the sampling of blood which is required in most studies to determine the amount of medicine in the body. At the end of use animals are humanely killed by an overdose of anaesthetic. Samples of various organs may be taken and examined microscopically to ascertain whether the potential new medicine has caused changes that would prevent administration to humans. Some animals may be able to be kept alive at the end of a study and re-used in a subsequent study providing they are not suffering and in a good state of health. This will reduce the total number of animals used.
Application of the 3Rs	
1. Replacement	Some potential side effects of drugs can be detected using non-animal methods and these will be utilised

State why you need to use animals and why you cannot use non-animal alternatives	by Sponsors. However, to understand how a potential new medicine may affect the function of vital organs, either directly or indirectly, it is still necessary to use a whole animal in which all organ systems are present and their physiology is intact.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals will be set after consultation with a statistician or other qualified person. The minimum number of animals is used that allows the study to reach its objective. Historical data will be used where possible to help with this decision.
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 are appropriate species to use for evaluating the potential side effects of new medicines on the function of vital organs. The physiology of the cardiovascular, respiratory and central nervous systems has been well characterised in this species and many drugs show similar effects in rats and humans. Mice may be used if for some reason it is a more appropriate species than the rat. Guinea pigs are a more appropriate species for use in certain studies on the heart and airways since they are more predictive of effects in humans. In all studies, adverse effects experienced by the animal and the number of animal per group will be kept to the minimum to achieve the objectives. Where animals receive multiple doses of a test medicine, sufficient recovery time will be allowed between successive doses. Animals will generally be housed in groups and provided with specific materials to provide enrichment. Any periods of restraint or single housing will be kept to the minimum required to achieve the study objectives. The number of blood samples and the volume of blood taken for measurements will be minimised by using a micro sampling technique, where possible, which also minimises the restraint required. Appropriate anaesthetics and analgesics will be used during and after any surgical procedures required.

Project 13	**Production of Medical Diagnostic Reagents**	
Key Words (max. 5 words)	Blood, agar plates, vaccine production	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	x	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 objective is to aseptically remove blood from a superficial vessel of horses and sheep. Wherever in the world clinical microbiological investigations are carried out, the characteristic appearance of bacterial colonies on agar plates, supplemented with horse or sheep blood is a universally accepted and recognised first stage of the isolation and identification process. Defibrinated horse and sheep blood – used daily in culture media in the microbiology laboratory, in diagnostic tests and other laboratory procedures. In virology the use of complement fixation diagnostic tests requires the presence of sterile animal blood cells to act as markers. In clinical pathology animal blood is used to manufacture whole blood standards and controls. Donor horse serum, a product of sterile blood, is also required for the manufacture of a wide variety of	

	vaccines for human and animal health.
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)?	Animal blood provides the growth factors for a wide range of bacteria and is therefore vital for rapid diagnosis of infection in humans and animals. Horse serum is a necessary component in production of some vaccines.
What species and approximate numbers of animals do you expect to use over what period of time?	Horse – 400 Sheep – 3000 Re-use over five 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?	Mild severity, no adverse effects. Re-use
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal blood is used in microbiology on a daily basis as a aid to diagnosis. The difficulty of replacing animal blood with a synthetic source is that, whilst the growth support of bacteria might be replaced chemically, the reaction of the cell envelope in haemolysis is vital in diagnostics. Therefore, although artificial blood is being developed, it does not carry the required growth factor for a wide range of bacteria and in addition would not define bacteria as haemolytic or otherwise.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We work closely in conjunction with our customers to ensure that annual forward forecasting of blood requirements is as accurate as possible. This helps us to maintain the correct number of animals, allowing for fluctuations in orders and periods of rest and recuperation as required by the individual animal.

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.	Horses are herd animals, interaction within a herd is very important for minimisation of stress. On arrival on the farms horses are quarantined in smaller social groups before being introduced into the main herds. During this period they form a bond together and this friendship group will be introduced to the herds together. Horses have a close relationship with humans which limits any stress during the process. Also being large not so many are needed to produce the required product. The farm employs a dedicated shepherdess. Sheep are quarantined in small social groups before being introduced to the flocks. This helps them to form a friendship bond and minimises stress. The need for absolute sterility in the use of blood for diagnostic purposes makes the choice of large healthy donor animals essential. In comparison with small animals, blood donation subjects horses and sheep to minimum stress and reduces the number of donors involved. Horses and sheep stay on the farm for many years and the 485 hectares of extensive grazing and housing allow for natural and stress free herd/flock environment and behaviour. Blood collection is best carried out by a large specialised company with proper facilities for caring for the welfare of the animals and monitoring their haematological status, preparing apparatus and with extensive quality control facilities to ensure a consistent and reliable product. The laboratory results of the percentage haemoglobin of each horse and sheep and their excellent condition shows that the blood volumes and frequency of withdrawal has no detrimental effect on the animals. PCV of each animal is measured after each donation in the laboratory and recorded by the farm. On arrival, new animals are quarantined in small

	groups, for approximately two weeks, prior to their release in the same small groups into the herd/flock system when they will become part of the donor rotation. These friendship groups often remain intact throughout the animal's whole life on the farm and make a big difference to the stress free environment that the farm is promoting. The welfare of the animals, their quality of life and our animal husbandry are the top priority. The animals have extensive grazing over a large area of approximately 485 hectares. The herd/flock system remains intact throughout the winter period, which is November to April, when the animals are housed in big barns and fed in surrounding large open yards. Animals have complete freedom of movement between the barns and the yards where food is continually available. All winter food and bedding is home produced (grass silage, whole crop silage, oats and hay). This helps to maintain a healthy diet at all times. Wherever in the world clinical microbiological investigations are carried out, the characteristic appearance of bacterial colonies on agar plates, supplemented with horse or sheep blood is a universally accepted and recognised first stage of the isolation and identification process.

Project 14	**Safety Evaluation of Substances Administered to Man**	
Key Words (max. 5 words)	Regulatory, Toxicity, Safety	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	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 overall aim of this project licence is to generate toxicological data to support all stages of drug development and the safety assessment of food and drink additives and supplements and other substances administered to Man. The data generated will support three main areas: • To characterise the tolerance, general and genetic toxicity and/or toxicokinetics of substances to support administration to man, marketing applications and post-marketing activities and to enable the conduct of studies performed under other licences or in other facilities. • To provide support to drug discovery by early characterisation of toxicology liabilities related to a specific compound, a pharmacological class or a chemical series to allow selection of optimal drug development candidates, refine discovery strategies and provide appropriate animal models to enable effective candidate	

	selection. • To characterise toxicities, understand mechanisms, identify biomarkers and develop animal models of toxicities occurring in animals and humans at all stages of drug development and throughout a product life cycle.
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 overall benefits of this licence is that the studies performed enable the safety assessment of substances, in most cases prior to their exposure to Man via the generation of high quality data that is acceptable to regulatory authorities and which enables decision making. With specific regards to pharmaceuticals, the data generated will support the development of safe, new medicines designed to improve the health and quality of life in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a five year period, it is expected that the following number of animals will be used on this project. 30,000 rats 30,000 mice 3500 hamsters 2500 rabbits 1500 dogs 1500 pigs
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 procedures performed e.g. the administration of substances and sampling of blood and urine will cause transient discomfort only. Any reactions to treatment will be closely monitored and should cause no more than Moderate severity. At the end of the study, the animals will be euthanized and subjected to a post mortem in order to assess any effects on tissues and organs. A small number of animals may undergo surgery. The administration of an anaesthetic and subsequent observation of sufficient depth of anaesthesia will be performed by competent persons in order to minimise animals dying from inadvertent overdose of the anaesthetic. Risk of

	infection post-surgery will be minimised by the use of aseptic surgical techniques. A regimen of post-operative care following the surgical procedure will be implemented which will include the administration of an analgesic to minimise pain and discomfort post-surgery.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is generally accepted that the way in which a substance is metabolised within a living body has a significant effect on how it works and its potential toxicity. Consequently, for the majority of pharmaceuticals it is imperative they are tested on living animals in order to assess for toxicity of tissue, organs and systems e.g. the cardiovascular, respiratory and reproductive systems following repeated exposure. The use of alternative methods, including the use of dead animals cannot, at this moment in time generate relevant data which supports the submission of safety data to international regulators. Alternative methods such as *in-vitro* techniques will be used as much as practicable to supplement the work involving protected animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals will be used, recognising the fact that reduction is not achieved by using too few animals to achieve the objectives of the study. For Regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to internal guidance on study designs to provide the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. Project specific variations are used as required.
3. Refinement Explain the choice of species	The selection of appropriate species (and strains within species) for use of regulatory studies is of

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.	paramount importance and much has been published concerning how such selections should be made in particular circumstances. There is also an ethical and legal obligation to use the least neurologically sensitive species practicable. It is not appropriate to select species solely on the basis of historical precedent and the result of this is that previously unused or infrequently used species may be required for the proper safety assessments of safety studies performed under this licence. Animals will be housed in conditions compliant with the Home Office Code of Practice. The procedures performed will be validated and established to cause transient discomfort only. Procedures will be performed the minimum frequency commensurate with achieving the study objectives.

Project 15	**Acute Toxicology -Chemicals**	
Key Words (max. 5 words)	Acute Toxicology Chemicals	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
	X	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 aim is to identify hazardous properties of chemical/preparations with respect to acute toxicity (including primary irritancy and skin sensitisation), in order to assess the potential adverse health effects following short-term exposure of humans and animals, either directly or via the environment. This will provide the information required for appropriate classification and labelling, recommendation of safe handling and transportation, and formulation of risk reduction strategies. The specific objectives are to determine: • Skin irritancy potential: The potential of a chemical/preparation to produce local irritation (inflammatory responses) following a single application to rabbit skin. Wherever possible reversibility of dermal responses will be investigated. • Eye irritancy potential: The potential of a chemical/preparation to produce local irritation following a single application to the rabbit eye. Wherever possible reversibility of	

	ocular responses will be investigated. • Acute oral toxicity: The toxicity of a chemical/preparation will be determined following oral administration. This may necessitate administration of different dose levels to different groups of animals. • Acute dermal toxicity: The toxicity of a chemical/preparation will be investigated following dermal application to the rat or rabbit. This may necessitate administration of different dose levels to different groups of animals. • Skin sensitising potential: The potential of a chemical/preparation to induce a skin sensitisation (delayed type hypersensitivity) response in the mouse will be investigated.
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 conduction of acute toxicology studies on new chemicals and preparations will allow identification of hazardous properties with respect to acute toxicity, as required by national and international legislation, in order to assess the potential adverse health effects following short-term exposure of humans and animals, either directly or via the environment. This will allow classification and labelling of products to recommend safe procedures for handling and transportation, and to formulate risk reduction strategies. The principle benefit of the Contract Research Service approach is to provide Sponsors with information for which they do not have the necessary facilities, expertise or capacity to do in house The company's commitment to perform *in vitro* tests provides the opportunity for these tests to be conducted in parallel with the *in vivo* tests, thereby improving knowledge of their predictive capacity.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, Mice and Rabbits over 5 years. Approximate figures: 11000 rats will be used in oral and dermal toxicity studies. 300 mice in oral toxicity studies and 27000 in Local Lymph Node Assay studies. 2350 rabbits in skin, eye irritation and dermal toxicity studies.
In the context of what you	Adverse effects are minimised by the incorporation of

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 vitro* screening tests into the programme. Possible adverse effects are signs of irritation in skin and eye irritation tests (moderate severity). Signs of systemic toxicity in oral and dermal toxicity studies (moderate severity). Acute oral toxicity studies conducted in accordance with the acute toxic class method and the Up & Down procedure, also dermal toxicity studies have a severe severity rating, signs of toxicity may be more common in these studies. The level of severity is severe in these studies. The expected adverse effects seen in animals used in skin sensitisation studies are skin irritation and systemic toxicity (moderate severity) in the sighting studies. Only mild severity is expected in the main studies. All animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is a requirement of national and international legislation to conduct safety evaluation studies using guidelines which are accepted globally. Non-animal alternatives will always be used where these provide sufficient information. If full replacement methods are not available, relevant and reliable alternative methods will be incorporated into tiered testing strategies to reduce and refine methods using animals. Skin corrosion tests on animals are not conducted as validated *in vitro* methods are available (OECD 430, 431 & 435). Corrosive or severe skin irritants will not be tested in eye irritation tests. There is currently no single non animal test that can be used to replace the acute oral toxicity test but where appropriate, non animal methods will be used in order to assess acute oral toxicity. Dermal toxicity testing will not be conducted in animals on corrosive test items. There is currently no validated *in vitro* method to replace the dermal toxicity test but testing in animals will conducted only when classification is not possible using other data.. There is currently no single non animal method available to replace OECD 429 Local Lymph Node Assay. Validated non-animal methods (OECD 442C, OECD 442D and h-CLAT may be performed in

	combination with consideration of other information in order to minimise testing on animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals required to satisfy the guideline to which studies are conducted will be used. Reduced LLNA, limit tests and single sex studies are used wherever possible. Negative control groups are avoided if historical data exists for commonly used vehicles. Positive control groups may be required for LLNA studies but historical data, checked every 6 months is used wherever possible. Wherever possible the number of animals used on skin and eye irritation studies is limited to two if testing in a third animal will not alter the outcome of the study.
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 species is defined in the relevant guideline. The most appropriate species, based on sensitivity and availability of background data, with the lowest neurophysiological sensitivity, is chosen. Licensees are trained and competent in the appropriate procedures and are familiar with signs of pain, discomfort or distress. Anaesthetics/ analgesics will be used after consideration of the specific requirement of the procedure. Wherever possible non animal screening tests will be incorporated in order to reduce harm and minimise animal numbers. Studies performed to “severe” severity protocols are scheduled to start early in the working week and day so that critical observation periods occur within normal working hours and the frequency of observations can be increased depending on the potential for increasing pain and distress.

Project 16	**Imaging Agents - Biodistribution For QC testing of Ceretec**	
Key Words (max. 5 words)	QC, Batch release	
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
	X	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 meet marketing requirements for quality control (QC) batch release and end of shelf life for Ceretec.	
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 batch release of safe and effective imaging agents to aid in the diagnosis, staging and management of disease. Technetium (99mTC) exametazime is a radiopharmaceutical sold under the trade name Ceretec, and is used by nuclear medicine physicians for the detection of altered regional cerebral perfusion in stroke and other cerebrovascular diseases, Epilepsy, Alzheimer's disease, forms of Dementia, Transient ischaemic attack, Migraine and tumours of the brain. It can also be used for the labelling of leukocytes to localise intra-abdominal infections and inflammatory bowel disease.	

	In the UK and EU5 (UK,Italy,Spain,France and Germany) alone, the patient procedure numbers for Ceretec exceed 100,000 per year.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 2 year duration of the project it is anticipated that no more than 80 rats will be required.
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 only potential adverse effects are from: Anesthesia which is used for the purpose of restraint during the radioactive IV injection. All animals recovering from anaesthetic will be monitored until fully recovered. Overall, deaths resulting from anaesthetic are uncommon (<1%), and will be minimised by ensuring correct dosing/application of anaesthetics, and by good maintenance of body temperature, e.g. by use of heated pads. The imaging agent Due to the tracer amounts administered, no adverse effects are anticipated The severity limit for this licence is Mild At the end of the study animals will be culled by a schedule I method followed by removal of organs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vivo studies are required for the batch release and end of shelf life studies to meet product specifications of Ceretec. This is required by Health Authorities to meet lot release specifications for the product. Justification for the removal of the animal biodistribution test from the lot release and end of shelf life testing of Ceretec, Cobalt Stabilised Ceretec and Methylene Blue Stabilised Ceretec has been submitted. It is intended that no in vivo QC batch release will be required after 2017.
2. Reduction	The number of animals required for each study is based on regulatory requirements to meet product

Explain how you will assure the use of minimum numbers of animals	release specifications. This is to ensure that the data is robust enough to reduce the need to repeat studies. Work within GE healthcare has determined, through the use of stastical analysis, that the use of 3 animals produces data that is not statistically significantly different when compared to data obtained from 6 animals. Therefore specifications state that all batch release studies are performed on 3 animals.
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.	Rodents are the lowest vertebrate group on which well characterised effectiveness and biodistribution studies have been performed. Therefore the justification for the use of rats is based on available literature evidence of the suitability of the species, and previous data generated in-house. All animals will be closely monitored post administration of the imaging agent and should any animal display deviation from normal health it will be euthanised by a schedule 1 method.

Project 17	**Production of antibodies, antisera and blood products**	
Key Words (max. 5 words)	Antibodies, antisera, blood, antigen	
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
	X	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 supply of specific antisera/antibodies and blood product supporting fundamental and applied research and the development and delivery of human and animal healthcare.	
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)?	This services provided under this project licence will supply in-vitro diagnostic reagents to laboratories, nationally or internationally and underpins fundamental and applied research for the development and application of new materials in support of human and animal healthcare.	
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle 200 Camelids (Llamas and Alpacas) 25 Pigs 50 Sheep 75	

	Goats 30 Equidae -(Horse and donkey) 10 Poultry (Chickens, Layers and Broilers) 100 Rabbits 75 Guinea-pigs 25 Rats 225 Mice 225
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?	All animals used within this project licence, apart from laying hens when we may need to identify the specific hen that has laid each egg, are housed in a low stress naturalised environment and animals are maintained as members of a social group. For the vast majority of animals, the protocols followed in this licence are minimally invasive and therefore the severity level will be mild and the animals will experience (>95%) no signs of ill health or malaise. However, there is always the possibility that an animal will experience an adverse reaction and where this happens the animal will be treated to alleviate the symptoms or it may need to be put down. Whenever possible animals will be rehomed after completing a study. On occasions the nature of the study requires that the animal be put down at the end of a study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alternative methods for antibody production are used whenever possible. There are, however, cases where only the whole animal immune response is adequate to provide suitable materials or information. For example, to model the effect of a potential vaccine: where there is a requirement for a broad-spectrum antibody response or to provide cells for in-vitro selection and antibody production. Wherever possible alternative sources of blood product will be used e.g. collecting samples from animals being

	put down for food production. Animals used for antibody production are immunised by injection with the material to which a response is sought and subsequently blood samples are taken, Antisera/antibody production and blood products supply
2. Reduction Explain how you will assure the use of minimum numbers of animals	Antisera/antibody production and blood products supply group sizes are based on the study requirements which are normally in the region of millilitres. This volume dictates the minimum number of animals required. However effective management and the use of established procedures/protocols keeps this number to the minimum.
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 the first instance the species selected for antisera/antibody production and blood products supply are the most relevant to scientific and logistical (e.g. sample volume) requirements of the project and within the constraints of the licence. If this can be fulfilled with one or more species e.g. sheep or cattle, then the species chosen will be for its ease of use (blood sampling & dosing) and therefore less stress to the particular animal. Procedures are performed by Personal Licence holders, who have the training, experience in animal handling and frequency of application of techniques necessary to ensure that these are carried out so as to cause minimum stress to the animals.

Project 18	Production of Blood Products for Scientific Use.
Key Words	Blood, Horse, Sheep
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

The aim of the project is to provide a regular source of fresh sterile donor blood products from horses or sheep. Clinical and Veterinary Laboratories use the animal blood as a nutritious supplement for the manufacture of culture media for the identification of microbial organisms.

Defined volumes of blood are collected from healthy donor horses and sheep at pre-defined intervals.

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)?

Microbiology/Pathology laboratories have established methods for the detection of pathogenic organisms by recognition of the growth characteristics and colony morphologies of the different organisms on culture media containing defibrinated blood. These classical microbiology methods are well established and although new media types are more readily available culture media products containing horse and sheep blood are essential for the diagnosis in microbiology/pathology laboratories

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

Approximately 300 horses and 600 sheep will be used 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 levels of severity? What will happen to the animals at the end?

The adverse effects will be mild. Procedures have been designed to limit the suffering to donor animals. This includes refinement of the bleeding equipment and facilities to ensure animals are less stressed, and ensuring the bleeding process is efficient and ensures as little physical damage to donor animals as possible.

Application of the 3Rs

Replacement

Classic microbiology methods rely on the availability of horse and sheep blood.

Reduction

Process have been established to ensure the minimum numbers of animals are used. Each animal is logged within our database and closely monitored to ensure it can provide the correct amount of blood keeping animal numbers and cost to a minimum.

Refinement

There are no alternatives to the animals selected. The bleeding facilities and equipment are refined to ensure the animals are less stressed and prevent physical harm. The bleed process has also been refined to ensure efficiency.

Project 19	Viral Vaccines (Batch Release)
Key Words	Vaccine quality control; Vaccine safety
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Vaccines intended for human use are very complex and extremely sensitive products. Before a vaccine batch is released into the market, it is a legal requirement that it is tested by an independent laboratory to confirm the manufacturer's results for quality and safety. In rare incidences of adverse reactions after vaccination, it may be necessary to test in animals in order to investigate whether a vaccine batch has been, for example, sabotaged or compromised by improper handling or storage.

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 work performed under this licence provides independent verification that vaccines released into the market are efficacious and safe. Vaccination is a highly effective method of preventing certain infectious diseases. Routine immunization programmes protect children from a number of infectious diseases that previously claimed millions of lives each year. For travellers, vaccination offers the possibility of avoiding some infectious diseases that may be encountered abroad. Public confidence in vaccines is crucial to the control of disease. This project is part of continuing efforts to maintain that confidence.

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

Approximate numbers of animals expected to be used over the course of the 5 year licence are 5000 mice and 7500 rats.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

In the absence of a scientifically validated in vitro test for vaccine potency, this licence permits vaccine potency testing by injection into animals to induce an immune response. At approximately 1 month after injection, each animal, in turn, is anaesthetised and blood obtained for testing for antibodies. The animal is then humanely killed while still under anaesthetic. The severity limit for this protocol is mild. No systemic adverse effects are expected. Local complications due to the route of administration are not expected or are expected to be very rare. Animals will be monitored for signs of local pain, local irritation or general discomfort. Animals will be observed regularly by trained staff for the first hours. Veterinary advice will be sought in cases of other variation from normality. Any animal showing signs of other adverse effects as a result of the regulated procedures will be humanely killed unless normality can be restored promptly using no more than minor medical treatment.

Application of the 3Rs

Replacement

Where regulatory requirements permit and there are alternative, scientifically validated in vitro tests available, these tests are always used. However there remain vaccines where the relationship between the in vivo and in vitro assays is either not established or established only within defined parameters, for example, for a specific vaccine from a specific manufacturer. In these circumstances it is necessary to retain the ability to perform in vivo potency tests as outlined in this licence.

Reduction

The number of animals is defined primarily by the regulations. Where possible animals are grouped to minimise the number of control animals needed.

Refinement

The animal species used are prescribed by regulatory documents. In vitro tests used to supplement or eventually replace in vivo tests are also specified in regulatory documents from the World Health Organisation and the European Pharmacopoeias. Such documents undergo continual revision to minimise the number of animals used while ensuring that the data generated are meaningful.

The protocols described in this licence have been developed over many years and are as a consequence considered to be the least severe means of achieving robust results.

Animals are monitored by experienced staff throughout the test, and any developing significant adverse effects are humanely killed at the earliest practicable time. Based Based on past experience this is expected to be very rare.

Project 20	Efficacy Pharmacology Assessments: Circulatory, Gastrointestinal and Endocrine
Key Words	Pharmacology, Circulatory, Gastrointestinal, Endocrine
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

The objective of this project is:

- To generate and report data from *in vivo* pharmacology tests for new medicines for proof of concept using models primarily involving the gastrointestinal, endocrine and/or circulatory system.
- To generate and report data from *in vivo* pharmacology tests for lead optimisation.
- To detect side effects and toxicity so that compounds can be eliminated from further development early on in the development process.
- To generate and report data from *in vivo* pharmacology tests for comparisons purposes with market leaders for improved efficacy and/or reduced side-effect purposes.
- To satisfy the regulatory requirement for biosimilar testing (including testing of such drugs for efficacy prior to seeking pre-marketing authorisation)
- To introduce and validate new methods.

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)?

In the case of medicinal products, achievement of the objectives of this licence enables the identification of candidates to progress into clinical testing and to pre-marketing authorisation. The information will also be used by scientists and responsible clinicians for selection of both starting and limit doses for clinical studies and to identify parameters which should be clinically evaluated. Without these

studies progression of new treatments to early human studies and to patients/marketing could not occur.

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

Over the 5 year life of this Project Licence, it is estimated that 2,700 mice, 3,400 rats and 500 non-human primates will be used.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The severity limits specified are considered to be the minimum commensurate with achieving study objectives. The severity limits that are specified as moderate under this licence are due to the induction of ulcers and surgical procedures, with this severity limit assigned to the anti-ulcer and growth hormone bioassay protocols respectively. The remaining protocols, Gastrointestinal transit test, anti-diabetic activity, effect of thyroid hormone, bleeding time test, arterial thrombosis model and primate haemophilia model are specified as mild. Where possible, such as for rodent bleeding time, the rodent arterial thrombosis model and the primate haemophilia model, the procedures are conducted under non-recovery anaesthesia The mild severity is likely to be due to procedural effects such as dosing and bleeding. With minimal adverse effects due to the test article as these are not the first in vivo studies to be performed. Efficacy studies are conducted early on in the drug development process, typically prior to regulatory safety studies. However, it is expected that preliminary data will be available, in particular pharmacokinetic data which will enable dose levels to be selected which have the potential to show efficacy, with minimal adverse effects. Animals will be humanely killed at the end of a study. If required, humane endpoints (documented within the licence) will be applied to animals used under the protocols specified in this licence.

Application of the 3Rs

Replacement

Several in vitro techniques such as receptor binding, in silico modelling and functional high throughput assays are used during early drug development and provide an early indication of the potential therapeutic properties of the drug. However, to fully assess the pharmacodynamic effects (effects of a drug on the body) of a new drug in vivo testing is necessary. Only in a fully operational circulating system can the drug’s distribution, metabolism and excretion, which may alter or intensify the efficacy or adverse effects of the new medicine, be fully understood. For these reasons animal models remain essential in the development of new medicines.

Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

Reduction

Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.

Refinement

Rodent models are adequate to complete the majority of studies types within this licence and as such the use of species at a higher level of neurophysiological sensitivity has been avoided. An exception to this is for the primate haemophilia model where assessments are carried out under non-recovery general anaesthesia. For this model only treatments against potentially life threatening or debilitating human diseases or their effects will be evaluated and non-human primates will only be used where the purpose of the programme of work cannot be achieved by use of animals that are not primates. Such drugs will have high specificity to human targets that are not present in other species. In addition the body system under investigation has a similarity to humans not seen in other species. This model will be used as an alternative to the conscious primate model for which pain and discomfort is an integral feature due to spontaneous bleeds, particularly around joints.

Where scientifically possible animals will be assessed under anaesthetic. However due to the effect of anaesthetic on certain body systems and the relatively short duration of anaesthesia, this is not often possible. For example, prophylactic or therapeutic time-course effects cannot be assessed in anaesthetised animals, Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

Project 21	Metabolism and Pharmacokinetic Studies
Key Words	Regulatory, Metabolism, Pharmacokinetic, Animal
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Investigations of metabolism and pharmacokinetics of xenobiotics (foreign substances) are carried out in animals (*in vivo*) as part of broader safety testing programmes in order to generate information to assess risk of adverse effects compared to beneficial effects resulting from exposure to a new drug and it's metabolites. The data acquired enables national and international regulators to decide if a new drug or chemical should be sanctioned for use in the public domain. These investigations are conducted with pharmaceuticals, agrochemicals, animal health and biotechnology products, biocides, food additives and industrial chemicals.

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 principal benefit of this project is the provision of data to facilitate sound regulatory decisions regarding selection of species for toxicology studies, and extrapolation to humans (for pharmaceuticals); setting of exposure limits (agrochemicals) and animals (veterinary products).

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

Over the 5 year life of this Project Licence, it is estimated that 16500 mice (plus 900 transgenic mice), 16500 rats (plus 300 transgenic rats), 150 hamsters, 30 guinea pigs, 300 rabbits, 500 dogs, 500 minipigs, 500 cynomolgus monkeys, 20 goats and 200 chickens may be used. Adult animals will be used in all protocols. Neonate and juvenile rats may also be used to assess how the metabolism of a novel test material differs from that in an adult animal. Data generated from these studies is used to support human dose level selection.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The majority of animals on these studies are expected to have little, or no adverse effects. A very small number of animals, may show more significant adverse effects. Humane endpoints will be adopted if animals show excessive effects. Animals will either be humanely killed at the end of a study and the carcass (or individual tissue) investigated for test compound residues. Alternatively, animals may be retained for re-use on a different study or, where appropriate, rehoming may be considered.

Application of the 3Rs

Replacement

At present there are no scientific and legally acceptable evaluations for the absorption, distribution, metabolism and excretion (ADME) of xenobiotics that will satisfy regulatory requirements other than the use of animals. However validated *in-vitro* tests are used to support selection or replace these, wherever possible.

Reduction

Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.

All studies are subjected to ethical review of the objectives and procedures, prior to commandment of the work. Without approval of these, the study would not be carried out.

Where possible, animals may be re-used on a number of non-related studies.

Wherever practicable, a combination of sample collections (eg excreta collections, blood sampling, terminal tissue sampling) from the same animal will be used, to reduce overall animal usage.

Due to increased sensitivity in analytical methods, techniques have been developed that allow smaller sample volumes to be used. This has led to the reduction of the animals used by allowing more samples to be collected from each animal.

Refinement

The majority of animals used during the course of this licence will be rodents. Adult animals will be used in all protocols; additionally, neonate and juvenile rats may also be used. Scientific opinion, including that of the regulatory agencies, indicates the use of one rodent and one non rodent species for many of the metabolism studies that are required.

The most appropriate non-rodent species will be selected with reference to studies including, but not limited to in-vitro cross-species metabolism, in-vitro toxicity studies, and pilot pharmacokinetic and safety assessment studies. The species selected based upon this information is the one which is predicted to align most closely with human in terms of sensitivity, receptor homology and metabolism. Where scientifically justified the minipig will be used in preference to dog or primate, however, there are limitations in the metabolic characterisation of this model. For example the liver enzymes and transporter mechanisms for xenobiotics are yet to be established.

The most widely used characterised second species for metabolism studies is the dog. When considering veterinary products, the drug may be specifically aimed at dogs, in which case it is obligatory to study the target species. Dogs are only used where the purpose of the programme of work can only be achieved by their use. Under this project licence the use of dogs will be limited to the support of human or veterinarian healthcare and will not be used in agrochemical, industrial chemical or food additive testing.

Studies involving non-human primates are only initiated where no scientific or feasible non-human primate alternative exists. Primates will only be used in the testing of pharmaceuticals for use in life threatening or debilitating clinical conditions in humans. It should be noted that in vivo and in-vitro testing will sometimes indicate that primates are the only practical animal model that can be used in metabolic studies to give an indication of what may be expected in man, in order to fulfil the requirements of international regulatory agencies for the purpose of investigating life-threatening or debilitating clinical conditions in humans.

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.

Individual studies will be designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

Project 22	Coccidia control methods in poultry and game birds
Key Words	Coccidia, Parasites, Control
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Coccidia are protozoan parasites of which damage the gut wall. In the poultry there are a number of species of coccidiosis and their effects vary from harmless right through to life threatening. Coccidia infect every poultry house worldwide, and the infectious oocysts are highly resistant to heat, cold and disinfectants. Because of this and the fact they are very prolific and capable of developing resistance in the gut phase of their lifecycle to chemotherapeutic treatments eradication is nearly impossible

Due to this coccidiosis is a one of the most important poultry diseases as it thrives in high population/ density farmed poultry which the poultry industry is based on. Around $90 million dollars in the US and $3 billion worldwide are spent each year on coccidiosis defence alone. Coccidiosis has a major effect the poultry industry due to decreased performance, morbidity and mortality and still costing an estimated $300 million US dollars in losses, worldwide

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)?

This is a service licence that aims to provide an ongoing service to customers, pharmaceutical companies. It aims to with the provision of supportive data for

regulatory purposes on the safety, efficacy and quality of vaccines and anti-parasitic drugs against coccidia. It also protocols test and confirm drug sensitivity and resistance to existing chemotherapeutic anti coccidials as well as evaluation of other, non-chemotherapeutic control systems that could be used commercially. As such this will have direct impact on the welfare of commercial poultry and game birds. Coccidia have complex life cycles and different sexual and asexual stages which cannot be totally replicated/ maintained in-vitro. Research into such parasites therefore has to be undertaken in their natural hosts. The strains can be also host-specific; therefore choice of species is dictated by the coccidian strain under study/therapeutic agent to prevent this strain

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

To ensure minimal use of experimental birds, coccidia are only passaged to maintain stocks of important strains of coccidia, such as fully sensitive strains, or reference strains. Most coccidia remain viable in a refrigerator for up to 3 months depending on species, or are able to be stored in liquid nitrogen, so passaging is only done when necessary thus reducing the number of birds used. This work is demand lead by the licencing need, it is estimated that 16,000 chickens, 2,500 turkeys 600 gamebirds and 20 ducks will be used

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The vaccines and anti-coccidial drugs have been around for a long time and new vaccines and drugs would be using similar methods so risk of adverse effects are minimal. Our challenge models have been used successfully for decades and have been refined over these years. The challenge levels we use are very accurate and can give the determined severity limit required. The expected severity limit would be mild to moderate

Application of the 3Rs

Replacement

Some replication of coccidia has been achieved in cell culture, but the levels are extremely low and it would be impossible to demonstrate the efficacy of products or grow suitable quantities of parasites. Coccidia like *Eimeria* are host specific, so the natural host animals have to be used

Reduction

To ensure minimal use of experimental birds, coccidia are only passaged to maintain stocks of important strains of coccidia, such as fully sensitive strains, or reference strains. Most coccidia remain in a refrigerator for up to 3 months depending on

species, or are able to be stored in liquid nitrogen, so passaging is only done when necessary thus reducing the numbers of birds used.

The numbers of birds used varies, and in some cases is guided by the minimum requirements of licencing authorities such as the European Pharmacopoeia and statisticians are consulted before new studies are initiated if further guidance is required.

Refinement

Coccidia like *Eimeria* are host specific, so the natural host animals have to be used. With over 80 years of experience and knowledge of coccidia we have a vast understanding of the interactions and effects of the parasites. This enables us to determine accurate challenge levels so we are able to; use the minimal number of animals, cause the least harm/severity to the animals whilst still achieving a sample effect and achieve statistically accurate results

Project 23	Reproduction Toxicology Studies
Key Words	Toxicology, Safety, Reproduction
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

The overall aim of this project is to establish any effects on mammalian reproduction following exposure to one or more pharmaceutical materials. The data generated will provide essential information regarding the potential risk of a pharmaceutical material when exposed to humans.

The studies performed will have one or more clearly defined objectives; these being:

1. To provide data which meets the known international regulatory requirements for the development and use of pharmaceuticals which have a potential benefit to human health.
2. To test for toxic effects/disturbances resulting from treatment from before mating (males/females) through mating, implantation and the early gestational period.
3. To detect adverse effects on the pregnant female and development of the embryo and foetus.
4. To establish the effect on fertility following single or repeated administrations of a pharmaceutical material.
5. To establish the effect on the foetus during the late gestational period, through birth to weaning and also on post-weaning growth and development including attainment of sexual function.
6. To investigate unusual or unexpected findings following administration of a pharmaceutical material (Mechanistic studies).

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 overall benefit of this project is that it supports the development of safe, new medicines to improve the health and quality of life of patients by generating high

quality data that is acceptable to regulatory authorities and enables internal decision making.

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

It is estimated that the studies performed under this licence will use 4200 rats, 2100 mice and 700 rabbits each year over a 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 levels of severity? What will happen to the animals at the end?

It is expected that approximately 75% of all animals used will experience no more than Mild, transient discomfort only with the remainder experience Moderate discomfort. At the end of each study the animals will be humanely killed and an internal examination performed in order to assess any effects on the internal organs and structure.

Application of the 3Rs

Replacement

It is generally accepted that the way in which a material is metabolised within a living body has a significant effect on how it works and its potential toxicity. Consequently, for the majority of pharmaceuticals it is imperative they are tested on living animals in order to assess for toxicity of tissue, organs and systems e.g. the cardiovascular, respiratory and reproductive systems following repeated exposure.

Alternative methods such as *in-vitro* techniques will be used as much as practicable to supplement the work involving protected animals. However, at this moment in time, the majority of alternative methods, including the use of dead animals cannot generate relevant data which supports the submission of safety data to international regulators.

Reduction

The numbers of animals used will be the minimum practicable to achieve the objectives of the study and allow meaningful interpretation of the data.

Refinement

The majority of studies performed will use the rat; however, in embryotoxicity studies only, a second mammalian species traditionally the rabbit has been required. Reasons for using rabbits in embryotoxicity studies include the extensive background knowledge that has accumulated, as well as availability and practicality. Where the rabbit is unsuitable a second rodent species, for example mouse may be acceptable and will be considered on a case by case basis.

Animals will be routinely monitored and great care taken to ensure that no animal will suffer unduly. The procedures performed will cause transient discomfort only and humane endpoints set to ensure that suffering is minimised as much as practicable.

Project 24	Measuring the Strength of Immunological Medicines
Key Words	Immunological, Medicines, Vaccines
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

This project relates to vaccines, serums, antitoxins and allergen products.

An application for marketing authorisation must be supported by technical reports detailing quality control of manufacturing processes, the efficacy of the product and any adverse effects associated with the intended product.

The objectives of the project are:

1. To quantify the capacity of a new medicinal product to elicit an antibody response from animals exposed once or on several occasions to the product. The data outputs will contribute to information relevant to the efficacy of the immunologically-acting medicine, posology and/or the stability of the product.
2. Optimisation of the dosing schedule required to elicit an appropriate level of antibody response to a medicinal product and provision of the blood samples required for working-up and validating the analytical method. The secondary objective must be achieved before the key objective can be addressed.
3. Post-authorisation quality control of medicinal product. These will be named in the Project Licence by amendment.

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)?

Achievement of the objectives of this licence enables safe development of candidates to progress into clinical testing and to marketing authorisation. Without these studies progression of new medicines to early human studies and to patients/marketing could not occur.

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

Over the 5 year life of this Project Licence, it is estimated that 5000 mice will be used.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Animals are not expected to experience any adverse effects as a result of administration of test substances. Sub-clinical immunological responses, such as mild local reactions, whilst unexpected, may occur at the injection sites. Humane end points as described in Appendix 1 will be applied. The animals will be humanely killed at the end of a study. Humane endpoints (documented within the licence) will be applied to animals used under the protocols specified in this licence.

Application of the 3Rs

Replacement

In vitro techniques are in common use in the development and industrial-scale production of immunologically-acting medicines. There are, however, no established *in vitro* methods that will model the sequence of immunological events leading to seroconversion and quantify the antibody titre of a previously naïve animal exposed to the active component of any vaccine.

The Project Licence holder will be actively looking for non-animal alternatives which will be implemented when validated and accepted. Resources such as the European Union Reference Laboratory for Alternatives to Animal Testing will be monitored.

Animal–based studies will not be used when an internationally recognised pharmacopoeia details a relevant and specific *in vitro* method of assessing the stability or strength of an immunologically-acting medicine or when the literature reports a replacement method accepted by a relevant regulatory authority.

Reduction

Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is used to strengthen the overall scientific quality and relevance of studies.

Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

Refinement

The mouse is an adequate species to complete these types of studies and the use of species at a higher level of neurophysiological sensitivity has been avoided. The

choice of species is based on continuity of data for direct comparison with previous projects.

Most procedures will be conducted using manual restraint. If non-manual restraint is used, the animals will be acclimatised to the restraint before the procedure is conducted.

Illness of animals may have the effect of suppressing immune responses and frustrating the purpose of this type of study. Dose levels chosen for studies authorised by this Project Licence are intended to cause no overt signs of systemic illness. All treated animals will be inspected by trained animal technicians at least once daily. Humane end points will be applied as appropriate (see E protocol).

This project licence does not include Part E protocols categorised as severe.

Project 25	Assessment of Bioaccumulation in Fish
Key Words	Bioaccumulation, Regulatory, Safety, Chemical, Pharmaceutical
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

To allow the identification of hazards associated with the manufacture, transport and use of industrial chemicals, agrochemicals, pharmaceuticals and biocides such that their possible adverse effects on the natural environment can be determined. This will allow regulatory authorities to classify and label these substances, recommend safe handling procedures, and impose risk reduction measures if required such that the benefits provided by the substances can be safely achieved.

Specifically this project will assess the potential for these substances to bioaccumulate in fish.

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 main benefit of this project is the development of data to support the risk assessment of chemicals such that any detrimental effects on the environment can be minimised.

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

A variety of fish species including rainbow trout, common carp and bluegill sunfish are expected to be used. The total number of fish used over the 5-year licence period is expected to be approximately 13,000.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The individual studies undertaken involve exposure of groups of fish to varying concentrations of the chemical to assess the bioaccumulation of the chemical in the fish tissue. Following on from the bioaccumulation phase, a secondary phase where the fish have no exposure to the chemical is undertaken in order to determine whether removal of bioaccumulated chemical occurs. The tests are designed to be performed at concentrations below those which are predicted to cause adverse effects on the fish and hence in the majority of exposed fish no adverse effects will be seen. Experience suggests that in less than 5% of exposed fish will any mild adverse effects occur. Any fish that are exhibiting adverse effects which are in excess of the mild severity level will be humanely killed as soon as possible to avoid unnecessary suffering. In order to measure the bioaccumulation within the fish, fish will be sacrificed on a regular basis throughout the study to enable chemical concentrations within the fish tissue to be determined. All fish that are killed during the study and any that remain at the end of the test will be humanely killed using a Schedule 1 method.

Application of the 3Rs

Replacement

Current regulations e.g. REACH, require the use of fish to assess potential environmental effects. Non-animal alternatives have not yet been sufficiently validated for acceptance by various regulatory authorities and hence cannot be used to replace animal testing in this context.

Reduction

The number of animals used in regulatory toxicology studies is specified in the relevant Test Guidelines and is the minimum that is sufficient to allow meaningful interpretation and submission to a range of regulatory authorities. The use of the specified numbers of animals ensures that the data generated will be acceptable to regulatory authorities and hence will minimise the need for subsequent duplication or supplementary testing.

Where possible the results of QSAR predictions, physico-chemical testing and non-animal tests will be used to aid in the prediction of bioaccumulation hence reducing the number of animals required to satisfy the regulatory requirement.

Refinement

The fish species used have been selected in accordance with the relevant Test Guidelines and the age ranges of the fish are such that they are of the lowest neurophysiological sensitivity that will allow evaluation of the specific endpoints.

The species selected are representative of wild species. The data generated is therefore designed to protect these representative species in the environment thereby minimising larger scale environmental effects of tested chemicals.

Any fish that are showing a significant departure from the animal's normal state of health or well-being will be identified and humanely killed.

Project 26	Standards in Virology
Key Words	Vaccine, Virus, Disease
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

The objective is to make the materials necessary to be able to test the quality and effectiveness of biological medicines, such as vaccines. These tests are essential to ensure the vaccines are safe and effective before being administered to humans.

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 systems and assays used to control vaccines are well established and of demonstrated effectiveness. The reference materials and working reagents that are essential to the provision of effective vaccines can currently only be generated by the use of animals. The materials made under this licence contribute to the control of the potency and appropriateness of the vaccines produced by manufacturers and form a central part of the process by which satisfactory and protective products are marketed globally. The consequences of using a vaccine of low potency or inappropriate strain are that it will fail to protect recipients and disease burden in the human population could increase.

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

Embryonated eggs domestic fowl 2000 Ferrets 100 Turkeys 60 Sheep 150 Rabbits 20 5 years

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Typically the majority of animals used are not expected to experience significant ill-effects. The overall severity for all but a small number of ferrets (up to 25 animals over the course of 5 years) will be mild. For the ferrets that will be infected with

virulent influenza, the severity may be moderate the outcome of these infections can be unpredictable and so animals will be monitored very closely, the animals will be treated with antiviral medication to limit the symptoms they experience due to the infection. Any animal that has any significant adverse effect will be humanely killed using an overdose of anaesthetic. For animals being immunised there may be some local irritation at the site of inoculations particularly where adjuvants are used (adjuvants are chemical compounds that increase the immune response to an inoculation). This will be monitored and advice from a vet will be sought if any adverse effect is seen. All animals used under this licence will be humanely killed at the end of the study, or earlier if it is deemed necessary for the welfare of the animal.

Application of the 3Rs

Replacement

Specific antibodies are required for use in tests to characterise viruses and evaluate viral vaccines. These can only be generated in protected animals.We are investigating more modern methods of producing antibodies in the laboratory, using bacteria, but research in these areas is still at an early stage and is not yet ready for use.

Materials prepared for evaluation of vaccines must be prepared on the same substrate as vaccines. Embryonated eggs are currently the most common way by which vaccines against influenza are manufactured.

Turkey blood is a critical component of assays used to assess viruses and viral vaccines and the immune responses they elicit.

Reduction

Use of ferrets: - there will be sharing of production with other organisations requiring sera to avoid replication.

Few animals are required to generate the sera that are needed, but experience has shown over the years that several animals must be immunised or infected to be sure that there are sufficient suitable sera. Between two and four ferrets will be used in infection experiments and the same number of sheep for immunisation with non-replicating antigens. The numbers of animals proposed is regarded as the minimum necessary to produce the desired result, based on the experience of many years.

We are aiming to survey vaccine manufacturers to ask if they can use less volume of sera to test their products, and if so we will be able to reduce the amount we provide and thus reduce the number of sheep used to make sera.

The number of eggs required is more predictable and is regarded as the minimum to assess the material needed to test vaccines. The volumes of blood from turkeys are those required based on years of experience.

Refinement

Ferrets are among the few animals other than primates whose response to infection with influenza reflects that of humans; both the immune response and the clinical signs closely resemble that seen in humans. Methods for observation of clinical signs have been developed for earlier recognition of onset of disease allowing earlier intervention with the use of medication to relieve symptoms or termination as appropriate.

We will use pain relief medication (non-steroidal anti-inflammatory, similar to ibuprofen used by humans) to relieve symptoms in all infected animals, and antiviral medication to reduce the clinical symptoms in animals infected with virulent influenza.

It is recognised that group housing is preferable for optimum well-being of ferrets and wherever possible they will be group housed. There are situations where single housing is required due to husbandry needs or for safety reasons (for example where two male animals would otherwise fight and potentially injure each other).There are also occasions where an animal has been infected with a virus and must be housed singly to avoid infecting other animals. In these situations wherever possible animals will be housed in cages in rooms with other ferrets.

We will also try to use animals that need to be housed singly for husbandry reasons for the experiments that require an animal to be isolated due to being infected with a virus. This will minimise the number of animals that are not group housed.

Sheep are required to generate the large amount of serum required for a reagent for global use. The effectiveness of alternative adjuvants has been and will continue to be explored with the aim of minimising or removing the need to use Freunds adjuvant. Mice are too small and the response too variable to make them a practical alternative.

Embryonated eggs are the least sentient animal available and are used at $\leq$ two thirds incubation whenever possible.

Project 27	Drug evaluation in pre-clinical oncology models
Key Words	Oncology, Cancer, Efficacy, Combinations, Pre-clinical
Expected duration of the project	0 year(s) 2 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

To assess pre-clinically novel anti-cancer therapies.

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 advancement of anti-cancer treatments (including but not limited to immunotherapeutics, small molecules) for the treatment of cancer in humans. Increased exposure (global) to refined pre-clinical tumour modelling.

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

Mouse (immunocompetent and immnunocompromised). ~800 2 months bridging PPL.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Expected adverse effects are those relating to the implantation, growth and treatment of subcutaneous or orthotopic brain tumours; however, the controls in

place are expected to will reduce the presentation of adverse effects. The severity level is moderate. All mice will be terminated at the end of the scheduled studies.

Application of the 3Rs

Replacement

Mammalian physiology is required to accurately model tumour development, its spread (metastasis) to other organs, and to model drug delivery, clearance and efficacy at the tested levels (its Pharmacokinetic/Pharmacodynamic properties).

Mice are the lowest species in which fully competent immune system (for immunotherapeutic testing), or a reduced / absent immune system (for human tumour testing) is in place (dependent upon specific mouse model) to alow comprehensive cancer modelling and efficacy testing.

Reduction

Numbers of animals used will be minimised by appropriately controlling and powering studies to maximise scientific outcomes and minimise the necessity for repeat studies.

Where relevant and available, the use of longitudinal imaging modalities will be used to reduce the numbers of animals used: for example, for an orthotopic model whose internal tumour dimensions cannot be measured, tumour growth would normally have to be characterised by timed terminations of multiple study groups; using imaging technologies, this could be assessed in a single cohort of animals by multiple image points.

Refinements to tumour ulceration grading mean less mice are prematurely terminated resulting in increased statistical power whilst actually having very little negative impact on animal welfare.

Refinement

The protocols carried out will result in the least suffering as (i) real-time imaging modalities facilitates early termination of models with primary tumour at an internal location (ii) general anaesthesia and analgesia will be administered to minimise pain, suffering and distress. e.g. any side effects linked to tumour implantation/surgery.

Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity as a result of tumour:drug interactions and to ensure the drug levels used are not associated with any cumulative effects.

All tumour implantations/surgical procedures will be conducted using suitable anaesthesia along with peri and post-operative analgesia.

Project 28	Regulatory Ecotoxicology
Key Words	Ecotoxicology, Regulatory, Safety, Chemical, Pharmaceutical
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

To allow the identification of hazards associated with the manufacture, transport and use of industrial chemicals, agrochemicals, pharmaceuticals and biocides such that their possible adverse effects on the natural environment can be determined. This will allow regulatory authorities to classify and label these substances, recommend safe handling procedures, and impose risk reduction measures if required such that the benefits provided by the susbatances can be safely achieved.

Specifically this project will assess the ecotoxicological effects of these substances to fish following a single (acute) or series of exposures (chronic).

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 main benefit of this project is the development of data to support the risk assessment of chemicals such that any detrimental effects on the environment can be minimised.

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

A variety of fish species including rainbow trout, fathead minnow, common carp, bluegill sunfish, sheepshead minnow and turbot are expected to be used. The total number of fish used over the 5-year licence period is expected to be approximately 120,000.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The individual studies undertaken involve exposure of groups of fish to varying concentrations of the chemical to assess the effect that the chemical may have on survival and/or growth of the fish. Adverse effects ranging from mild discomfort through to death are expected during the course of this project. However in the majority of exposed fish adverse effects will only be mild. The programme of work will be designed in accordance with the principles of the 3Rs in order to minimise animal use and severity of procedures. Tiered testing strategies will be implemented, so that the results of one study can be used to refine the remaining studies in the programme thus minimising the severity of any adverse effects. All fish that are exhibiting significant toxic effects, and those surviving to the end of each test, will be humanely killed as soon as possible to avoid unnecessary suffering.

Application of the 3Rs

Replacement

Current regulations e.g. REACH, require the use of fish to assess potential environmental effects. Non-animal alternatives have not yet been sufficiently validated for acceptance by various regulatory authorities and hence cannot be used to replace animal testing in this context

Reduction

The number of animals used in regulatory toxicology studies is specified in the relevant Test Guidelines and is the minimum that is sufficient to allow meaningful interpretation and submission to a range of regulatory authorities. The use of the specified numbers of animals ensures that the data generated will be acceptable to regulatory authorities and hence will minimise the need for subsequent duplication or supplementary testing.

Where possible the results of QSAR predictions, physico-chemical testing and non-animal tests will be used to aid in the prediction of toxicity hence reducing the number of animals required to satisfy the regulatory requirement, e.g. by performing Threshold tests

Refinement

The fish species used have been selected in accordance with the relevant Test Guidelines and the age ranges of the fish are such that they are of the lowest neurophysiological sensitivity that will allow evaluation of the specific endpoints.

The species selected are representative of wild species. The data generated is therefore designed to protect these representative species in the environment thereby minimising larger scale environmental effects of tested chemicals..

Any fish that are showing a significant departure from the animal’s normal state of health or well-being will be identified and humanely killed.

Project 29	RODENT TOXICITY, TUMORIGENICITY AND SAFETY STUDIES
Key Words	Regulatory, Rodent, Safety, Toxicity, Tumorigenicity
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

The project aim is the determination of scientific and/or regulatory endpoints in rodent toxicity, tumorigenicity and safety studies for submission to regulatory authorities and/or for safety assessment purposes.

Regulatory approval is required to allow a new drug to be tested in human or veterinary trials, or for a chemical, agrochemical, food additive/substance or medical device/article to be marketed.

Studies are designed to determine specific toxicity or regulatory endpoints, and/or for safety assessment, ranging from single dose to 12 month repeat dose toxicity studies and life-span tumorigenicity studies (which determine any changes in tumour profile). Tumorigenicity studies can also be performed over a shorter period using genetically altered mice and by using a short study method. Other protocols include the provision of body fluid/tissue samples, use of juvenile animals exposed in the womb, and validation/research and development studies.

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)?

Governments require (and the public expects) that substances we are exposed to are safe or that their potential hazards are well understood and documented. The data generated from the studies performed under this project will be used to inform decision-making processes on substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain clinical trial approval, marketing authorisation or product registration. This safety assessment is of immense importance along with other non-rodent and non-animal studies in demonstrating to governments and the public the safety of these substances or highlighting their known hazards and safe handling.

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

Rats: 111,600 Mice: 68200 Hamsters:10300

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Administration of test substances may result, usually at the highest dose level in mild to moderate signs of toxicity, eg mild/moderate effects on food consumption, bodyweight, clinical pathology parameters, neurobehavioural parameters and organ function tests. Studies involving therapeutic agents (i.e. pharmaceuticals, biologicals and veterinary/animal health products) may produce pharmacological or pharmacodynamic effects at all dose levels, but they are expected to be transient. Experience shows that the majority (~65%) of animals are not expected to show any clinical signs of suffering (either no clinical signs or normal background signs expected of the rodent strain). A small percentage (~15%) may show transient subtle to mild clinical signs. Moderate signs of adverse effects may be seen in some animals (~20%), usually in the higher dose groups. Lethality and/or severe effects are not study objectives in any of the protocols within this licence, but for preliminary studies that may be the first animal studies with limited data available, a very small percentage of animals may inadvertently show severe findings before they are immediately and humanely killed. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate distress due to, for example, withdrawal of blood.

Application of the 3Rs

Replacement

There is currently no regulatory and scientifically acceptable alternative to the use of rodents in these studies.

Reduction

The regulatory guidelines usually indicate the number of animals in a study; otherwise, the number used is the minimum to achieve the aims of the study.

Refinement

The regulatory guidelines require toxicity testing in rodents, with the rat, mouse and less commonly, hamster being regulatory accepted test species.

Studies are performed in a stepwise manner, starting with preliminary studies using small numbers of animals where there is limited information. This gives the highest prospect of refining and optimising the programme to achieve the desired scientific endpoints and also resulting in the least pain, suffering, distress or lasting harm in the animals.

All animals are regularly monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, or humane killing of affected animals).

Project 30	Antibody Production for Research, Diagnosis and Therapy
Key Words	Antibodies, sheep, goats, hens, donkeys
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Farm animals (sheep, goats and hens) and donkeys will be injected with minute quantities of proteins, peptides or haptens conjugated to a carrier protein to stimulate the production of antibodies. Blood donations will be taken and the antibodies from the blood serum will be used to produce antivenoms to snake bites, or treatments for other life-threatening conditions such as serious infections or drug overdose. Antibodies are also required for use in a vast range of laboratory tests for the diagnosis and monitoring of disease.

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)?

Research projects for advancement of science, improved reagents for diagnosis and potential treatment. Early stage development of novel therapeutic treatments for diseases such as Clostridia, Leishmania, snake bites, Ebola, colchicine poisoning, CPG (cancer marker for novel cancer therapy - DNA oligonucleotides containing unmethylated deoxycytidylyl-deoxyguanosine dinucleotides are commonly referred to as CpGs Identification of improved antibody reagents for human, animal and plant diseases, e.g. malaria, drugs of abuse, legionella, ricin, and botulinum. There is a great unmet demand for antivenoms, especially in the third world, where every year many thousands of the poorest people and their children die from snake bite or suffer serious consequences such as amputations. Antivenoms are the only effective treatment for snake bites or many other envenomations. However, there is currently a crisis in supply, particularly for Africa. The challenge is to provide affordable treatments for the populations at most risk.

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

Up to about 250 sheep, 10 goats, 2 donkeys and 20 hens will be used each year.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Only mild procedures are used for injection of the animals. The collection of their blood is comparable to blood donations by humans. Antibodies from hens may be obtained by collecting their eggs, without need for bleeding, because hens' antibodies are found in their egg yolks as well as their blood. Sheep and goats, with their contractile spleens can adapt to regular bleeding, with no effect on their health. Unlike many other species used for antibody production sheep and goats are genetically programmed for “affinity maturation” of the antibody response; leading to much higher antibody affinity than other species. Donkeys pose a problem because, like horses, a vigorous local immune and inflammatory response may lead to swelling and a sterile abscess at the immunisation site. Donkeys may also show wide individual variability and some may show substantial reaction to injections. Only Freund’s Incomplete Adjuvant will be used in donkeys. Less than 1% incidence of systemic reactions, e.g. anaphylactic shock, is expected or anaemia. During the previous 5 years of the licence no systemic reactions have been observed. A mild severity level is expected in animals used in short projects and this can become moderate severity in animals used for longer projects.

Application of the 3Rs

Replacement

There are still applications for which animal derived antibodies are essential, e.g. antivenom production, where antibodies to all of the epitopes of the toxins and peptides are required: 10s to 100s. It has been proven that using large farm animals, e.g. sheep and horses, commercial production for human therapy of life threatening diseases is reliable and cost effective: products should be, safe, effective and affordable at their point of use.

Donkeys need to be used to produce the secondary reagents, i.e. donkey anti-sheep IgG, Fc, / Fab. These reagents cannot be produced in sheep as they would not be recognised as foreign and hence not produce an antibody response. Donkey secondary reagents are used in assays which employ a sheep antibody as the primary reagent.

Reduction

Re-use, providing there are no scientific or welfare prohibitions, reduces the total number of animals used in regulated procedures. Intelligent up front design and selection of the antigen can have a large positive effect on the desired antibody response and thus limit the numbers of animals required in order to obtain the required results.

Ig has an on-going research programme with partners to develop methods for sheep monoclonals and phage display antibodies through purification of peripheral blood lymphocytes from hyper-immunised sheep. These two projects have the potential to reduce the future number of animals as once immortalised antibodies can be produced in-vitro and the use of further animals is reduced or eliminated.

Refinement

The phylogenetic distance of hens from mammals may lead to raising of superior antibodies against some mammalian proteins, which may have advantages for certain diagnostic applications.

Donkeys are employed for the production of second antibodies only. Second antibodies must be produced in a species distant from the host used to produce the primary antibody.

Antibodies produced in animals ("polyclonal" antibodies) are the most effective because of the power and exquisite specificity of the immune system. "Monoclonal" antibodies may be produced in the laboratory but they are not as effective as polyclonals for these purposes, and are more expensive.

Sheep and goats provide the most effective and economical source of polyclonal antibodies for antivenoms and other therapeutic uses. They may be bled regularly with no ill effects. Polyclonal antibody production must be initiated in order to provide the B-cells or RNA required for in-vitro techniques such as monoclonal or phage display antibody production.

The use of animals such as sheep, goats, donkeys and hens permits the husbandry of animals under high standards of farming conditions; with grazing in fields in the summer and indoor housing with feed supplements in winter. Their useful life is generally longer than commercially farmed animals. All animals are under the day-to-day care of an animal welfare officer and are visited frequently by an external veterinary surgeon. Refined techniques include optimised emulsion preparation, targeted immunisation sites, breed selection (hybrids not pure breeds, larger breeds).

Project 31	Toxicity in Macaques by Inhalation Administration
Key Words	Pharmaceutical, Regulatory, Primate, Inhalation
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

This project enables the programme of regulatory, immunology and toxicology studies by inhalation exposure in macaques, and the validation or investigative studies which enable the regulatory programme.

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)?

New medicines have the potential to benefit in new or improved disease treatments. Before potential new medicines are administered to humans their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to people taking new medicines. At present there are no alternatives that don't use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

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

Macaques (cynomolgus and rhesus) 1150 over 1 year.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Procedures carried out during these studies include: : Dosing (eg inhalation administration) : Blood sampling or collection of urine for measurement of different components as changes in these may serve as early indicators of toxicity. Doctors for similar reasons often take blood and urine samples from humans. : ECG monitoring to assess changes in heart function (e.g. number of heart beats per

minute). This technique is also used by doctors to assess heart function in humans. : Examination of the eyes using a similar device to that used by opticians : Examination of more unusual parameters, eg retinography, seminology (sampling by direct stimulation), body temperature by rectal thermometer (such as a doctor might use for a small child). A degree of restraint or confinement may be required for some procedures. The animals are trained using positive reinforcement (treat rewards for compliance) to move about the cages for handling/procedures, and to sit in restraint chairs. Animals are acclimatised to mask and chairs and then during study animals are restrained in chairs and masks attached to nose and mouth area for a period of time. Most animals adjust to this and will sit comfortably for the duration of inhalation. Some animals become agitated and these are comforted by stroking to which they respond. Rarely some animals may become so stressed/or breath hold they collapse and as soon as this is detected animals are removed from the mask and recovered. These animals may be removed from treatment altogether. Animals are given a treat when returned to home cage. Some animals are re-used, but most animals are humanely killed at the end of the study by an overdose of anaesthetic to allow detailed examination of the organs. The majority of animals are expected to have mild adverse effects such as slight weight loss. A small percentage of animals may show more significant adverse effects e.g. more marked weight loss, or changes in appearance or behaviour (e.g. reduced activity) indicative of moderate severity. Humane end-points are applied as specified in the PPL.

Application of the 3Rs

Replacement

Pharmaceutical testing is a mandatory legal requirement and provides information on risks to people taking new medicines. At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

In vitro and in silico methods are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace in vivo studies.

We maintain a constant awareness of regulatory guidance and ensure that where non-invasive methods exist which fulfil the regulatory requirement they are used in preference to animal studies.

Reduction

The numbers of animals used in any particular study are generally linked directly to those indicated in the published regulatory guidelines. Animal numbers are kept to the minimum commensurate with meeting the objective of each study.

Refinement

We use non-human primates when other species (dogs and/or pigs) are unsuitable by one or more of the following criteria:

: kinetic or metabolic differences from man;

: species specific pharmacological or toxicological response,

Or when only primates are suitable by the following criteria:

: relevant toxicity or pharmacology which is only shown in a primate

: study design requires assessment of effects on organ systems or receptors for which primates are the only relevant model.

All procedures are subject to ongoing assessment and technique improvement and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and veterinary staff are on call at all times to assess and ameliorate adverse events.

Refinements to improve the animals experience include but are not limited to group housing, environmental enrichment, including novel toys and foods, human interaction, acclimatisation and training to procedures, to move around the cage and to leave the cage voluntarily as required, forage opportunity and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint