Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 3

Projects with a primary purpose of: Regulatory Use/toxicity

Project Titles and keywords

1. **Polyclonal Antibody, Normal Serum and Antigen Production**
    - Antibody, Serum, Antigen

2. **Testing of Veterinary Immunologicals**
    - Vaccines, Potency, Veterinary

3. **Non-Regulatory Studies**
    - Non-regulatory, validation, methodology, development

4. **The development of novel and improved viral poultry vaccines**
    - Poultry Viral vaccines

5. **Testing of Veterinary Immunologicals**
    - Vaccines, Potency, Veterinary

6. **Ex-Vivo Modelling of Cellular Pharmaco-Metabolism**
    - Cell preservation, hypothermia

7. **Detection of genotoxic substances**
    - Genotoxicity, Mutation, DNA Damage

8. **In vivo DMPK/ ADME profiling and screening in rodent models**
    - Metabolism, absorption, distribution, excretion, pharmacokinetics

9. **Metabolism of Chemicals**
    - Chemical, REACH, Residues, Metabolism

10. **Development of Veterinary Pharmaceuticals**
    - Veterinary, Safety, Residues, Efficacy

11. **Pharmacokinetics of Pharmaceuticals**
    - Pharmaceutical, Absorption, Distribution, Metabolism, Excretion

12. **Novel delivery systems for pharmaceutical agents**
    - Pharmacokinetics, bioavailability, tolerability

13. **Safety Pharmacology**
    - Safety, pharmacology, telemetry, regulatory

14. **Safety Pharmacology Assessments**
    - Safety Pharmacology

15. **Environmental toxicology and metabolic fate**
    - Environment, safety, pollution, chemical, pharmaceutical

16. **Pyrogen Testing**
    - Pyrogen Testing, Rabbit

17. **Toxicological evaluation of novel therapeutics**
    - Toxicology, dose range finding, safety, side effects, carcinogenicity

18. **Toxicology of Pharmaceuticals**
    - Pharmaceutical, Toxicology, Safety, Preclinical, Nonclinical

19. **Toxicology of Chemicals**
    - Chemical, Toxicology, REACH

Project 1	**Polyclonal Antibody, Normal Serum and Antigen Production**	
Key Words (max. 5 words)	Antibody, Serum, Antigen	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	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 production of antibodies and antigens using animals is required by UK based Companies that manufacture Diagnostic Test Kits for the detection of disease in man and Pharmaceutical Companies for the production of vaccines. Diagnostic test kits are used by Blood Banks and Hospitals throughout the world for the detection of common bacterial and viral diseases in man that include Meningitis, Hepatitis, MRSA, Syphilis, Influenza, Salmonella, Shigella and Streptococcus infections. The key component of many diagnostic test kits are antibodies and antigens specific to the infecting agent, currently there are no methods available for the production of specific polyclonal antibodies using non	

	animal alternatives, similarly the growth of certain bacterium such as the one causing Syphilis cannot be achieved with tissue culture techniques. The majority of diagnostic manufacturers require normal animal sera for the dilution of antibodies and control components.
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 real value of diagnostic kits is the **rapid** diagnosis of infection so that appropriate treatment can be given <u>immediately</u>. The use of appropriate diagnostic tests is part of a progressive effort to minimise pain, stress and discomfort in man
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep and Goats are commonly used exclusively for the production of polyclonal antisera. Alpacas and Llamas are used to provide a unique type of antibody. A maximum of 574 animals per annum 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?	Animals used for antibody production will be dosed with antigen over varying periods depending on the antigen, blood sampling will take place at intervals for the purpose of assessing antibodies, in all cases the final samples will be taken under general anesthesia. The procedure is classified as **Mild** Animals are monitored at all stages of the processes to limit adverse affects, dosing is reduced or omitted if there is a concern that further inoculations may cause distress to the animal, distress would normally be exhibited by reduced food and water intake. Each animal is monitored for early signs of reaction to the antigen.

	On completion of each schedule of work blood will be harvested under terminal anesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Currently there are no methods available for the production of specific polyclonal antibodies using non animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reductions of 30% have been achieved in the last 12 months by maximising techniques for the recovery of blood and processing in the Laboratory, this initiative will be continued. Future plans include using purpose bred animals that produce larger volumes of serum due to their size and weight (purpose bred strains). A further 25% reduction of animal use is expected with this approach.
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.	Sheep and Goats have been historically used for antibody production, they were originally chosen for their ease of use (blood sampling & antigen dosing), plentiful supply and ability to produce high quality antibodies. Refinement is achieved in many ways including; use of disease free stock, an ongoing training / coaching system of staff to ensure good welfare, environmental enrichment, objective health monitoring.

Project 2	**Testing of Veterinary Immunologicals**	
Key Words (max. 5 words)	Vaccines, Potency, Veterinary	
Expected duration of the project (yrs)	3 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)	This work will ensure that we can manufacture and market several different farm-animal vaccines that are consistent, safe and reliable.	
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)?	Vaccines protect animals against serious and potentially fatal diseases. The vaccine formulations have been used for several decades and reliably provide excellent protection from disease. Use of these products worldwide is established veterinary practice and enhances the welfare of animals by controlling animal disease or human food poisoning. Laboratory testing is required to ensure consistency of the vaccine and ensures that any substandard product is not released for use. Greater than 500 million chickens, sheep, pigs and cattle will be protected from disease by the vaccines tested under this project.	
What species and approximate numbers of animals do you	Approximate numbers of animals expected to be used over the course of the 3 year licence are:	

expect to use over what period of time?	rabbits -3000
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 cause little discomfort to the rabbits and no adverse effects are noticed. The severity of the procedure is mild and the animals are euthanised at the conclusion of the tests.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where statutory requirements permit and there are alternative tests available, these tests are always used. Working with animals is only where specifically stipulated by regulatory requirements or for the production of antisera required for the *in vitro* tests required by pharmacopoeias and/or marketing authorisations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals is defined by the regulations to give a valid test for the vaccine; by making as much vaccine as possible in one batch the minimum number of animals are used for potency testing.
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 for animal testing is dictated primarily within the European Pharmacopoeia. These are established tests which provide reliable results enabling the manufacture of vaccines working with the minimum number of laboratory animals. In recent years we have had success in replacing some of the tests working with animals with laboratory assays and are continually striving to, and have invested in methods to allow us to move away from animal testing wherever possible. All testing is performed in our own facilities and animals are cared for by fully trained, experienced and competent animal carers who are supported by designated veterinarians experienced in laboratory animal care and medicine. The potency tests are mild, causing little discomfort to the

	animals, however in the event of an adverse effect being observed medical intervention or humane euthanasia will reduce the possibility of the animal suffering.

Project 3	**Non-Regulatory Studies**	
Key Words (max. 5 words)	Non-regulatory, validation, methodology, development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	✓	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		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 principal objectives of the project are to establish new methods for the administration of materials or the withdrawal of biological fluids from protected animals with the aim of refinement and where possible, reduction of the numbers of animals used.	
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 success of regulatory studies which assess the safety of a material if exposed to Man or animals is very much reliant on the use of validated techniques which are capable of generating accurate results consistently. Studies performed under this licence will refine techniques and establish methodology in order to determine best practice for use on regulatory studies. The work performed will also ensure that future regulatory studies can be performed in compliance with Good Laboratory Practice standards.	
What species and approximate numbers of	Over a five year period approximately 1000 rats, 1000 mice, 350 hamsters, 500 rabbits, 50 dogs and	

animals do you expect to use over what period of time?	350 pigs may 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?	It is expected that the majority of animals will suffer no more than Mild discomfort. Where Moderate severity is reached then it will be short-lived. Animals will not be dosed with materials known to be toxic to them. On occasions however, it may be necessary to dose the animals with a material that causes a known, but transient effect – for example, a material that increases heart rate. This may be necessary when validating equipment that will be used to monitor activity in the heart and the objective is to determine if the equipment is capable of detecting changes in the rhythm. In most cases the animals will be painlessly killed at the end of the study; however, if appropriate they may be considered for re-use on future regulatory safety studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The type of data necessary to achieve the objectives of this licence can only be obtained from live animals. The data for inclusion in biological databases for example, which may include growth rate, food consumption and blood profiles, or physiological data used for the validation of equipment cannot be produced by alternative methods. Equally, the development or refinement of technical procedures can only be assessed as suitable for purpose if they are conducted on live animals. Without the use of animals it would be difficult to assess levels of severity experienced by the animals during their performance. Some of the work conducted under this licence will support the validation of *in-vitro* techniques which in time may assist with the 3R's.
2. Reduction Explain how you will assure the use of minimum numbers	There are no specific guidelines relating to the minimum number of animals required for non-regulatory studies; Consequently, the number of

of animals	animals required will be determined on a case by case basis and typically by the objectives of the study. Rodent studies designed to provide background to support regulatory carcinogenicity studies will use no more than 100 males and 100 females, which is considered to be the minimum necessary to provide the required data on tumour incidence, taking into account the very low incidence of some spontaneously occurring tumours. For other types of background data studies, the numbers of animals used will, where possible, be determined by statistical power analysis using an assessment of variability of the most variable parameter to be assessed. Where the data are of a type which does not allow standard power analysis, a statistician may be consulted to determine the minimum number of animals required to meet the objectives of the study. Where the objectives of a study are considered not to be affected by the sex of an animal then the numbers of animal used may be reduced by using one sex only.
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.	One of the primary purposes of this licence is the refinement of procedures. The selection of appropriate species (and strains within species) for use on regulatory studies is of paramount importance and much has been published concerning how such selection 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 purely 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 assessment of safety in pre-clinical studies. The species will be selected according to the regulatory requirements for the studies to be supported by the validation or background data studies. In the majority of cases the rat or mouse will

	be used, but where there is justification the hamster, rabbit, dog or minipig may be used. Animals will be housed in compliance with current Home Office code of practice. The procedures undertaken are expected to cause only transient levels of pain or distress and will be performed the minimum frequency necessary to achieve the study's objectives. Clinical observations will be performed routinely on all animals by competent technical staff. Any animal found to be in undue pain or distress will be euthanised.

Project 4	**The development of novel and improved viral poultry vaccines**	
Key Words (max. 5 words)	Poultry Viral 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)	The development of new viral vaccines for poultry that are safe, efficacious and easy to administer.	
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 than 70 billion chickens are produced globally every year and in the absence of vaccination these remain susceptible to a wide range of common poultry virus diseases including infectious bronchitis, Marek's disease, infectious bursal disease and Newcastle Disease. Improved vaccination will help to reduce the impact of such diseases and enhance the welfare and general health of farmed poultry, thus increasing the availability of safe, affordable poultry meat and eggs	
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 19,500 birds and 15,000 embryonated eggs are expected to be required over the five year term of this licence	

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?	*Following exposure to disease, unvaccinated birds or those treated with a poorly efficacious vaccine may become severely ill and potentially die. Careful selection of the amount of virus to be administered and frequent observation of the birds is intended to minimise this risk.* Birds will be humanely euthanased 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	Non animal based systems are not available for the production of all chicken viruses which instead typically require the use of fertile chicken eggs. The use of chickens to demonstrate vaccine safety and efficacy is specified by the appropriate licensing authorities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals required for regulatory studies is dictated by the appropriate guidelines. Where possible however experiments will be designed to combine the collection of data on as many different parameters as possible within a single study. A qualified statistician will provide advice to help to determine the minimum group sizes required to generate statistically meaningful results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	It is only possible to demonstrate the safety and efficacy of a vaccine in the species for which it is intended, in this case poultry. All studies are planned in advance and the experimental design reviewed and refined by discussion within the scientific team. Further refinement is encouraged through briefings with all individuals involved in the care and welfare of the animals both before and after completion of the study. Clearly defined humane end points will be defined and where the disease is more severe or a novel isolate of unknown severity is used the frequency of observation will be increased during any anticipated critical period. Birds will be euthanased immediately the humane end point has been reached.

Project 5	**Testing of Veterinary Immunologicals**	
Key Words (max. 5 words)	Vaccines, Potency, Veterinary	
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)	This work will ensure that we can manufacture and market several different farm-animal vaccines that are consistent, safe and reliable.	
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)?	Vaccines protect animals against serious and potentially fatal diseases. The vaccine formulations have been used for several decades and reliably provide excellent protection from disease. Use of these products worldwide is established veterinary practice and enhances the welfare of animals by controlling animal disease or human food poisoning. Laboratory testing is required to ensure consistency of the vaccine and ensures that any substandard product is not released for use. Over 900 million chickens, sheep, pigs and cattle will be protected from disease by the vaccines tested under this project.	
What species and approximate numbers of	Approximate numbers of animals expected to be used over the course of the 5 year licence are:	

animals do you expect to use over what period of time?	mice -70200 rabbits -7100 guinea pigs- 7800 chickens -2020 calves-20 sheep - 100
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 tests classified as mild severity cause no adverse reactions and no discomfort to the animals. The majority of the tests in rabbits and chickens fall into this category. Sheep and cattle are not expected to show clinical signs as a result of their vaccinations within this project. Therefore, once they have been observed for the required time to ensure vaccinal efficacy, they will be released from the act and returned to an agricultural premises. Some tests are classified as moderate severity because live bacteria are administered. This may cause lack of mobility breathing problems or a rise in body temperature. Animal will be closely monitored during these periods. A few of the tests are severe as they are assessing toxicity or inoculation of virulent cultures. On these protocols a percentage of the animals will die or be humanely euthanised as soon as clinical signs appear. Staff are well trained in the onset of the symptoms in the severe protocols and, where early intervention allows, the end of the test will be brought forward. Animals other than sheep and cattle are euthanized at the conclusion of the tests.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where statutory requirements permit and there are alternative tests available, these tests are always used. Working with animals is only where specifically stipulated by regulatory requirements or for the production of antisera required for the *in vitro* tests

	required by pharmacopoeias and/or marketing authorisations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals is defined by the regulations but where possible testing is grouped to minimise the number of control animals used. Vaccine production is planned to ensure the least possible number of batch safety tests will be undertaken for release of product to non EU countries.
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 for animal testing is dictated primarily within the European Pharmacopoeia. These are established tests which provide reliable results enabling the manufacture of vaccines working with the minimum number of laboratory animals. In recent years we have had success in replacing some of the tests working with animals with laboratory assays and are continually striving to, and have invested in methods to allow us to move away from animal testing wherever possible. All testing is performed in our own facilities and animals are cared for by fully trained, experienced and competent animal carers who are supported by designated veterinarians experienced in laboratory animal care and medicine. The tests are relatively predictable and enables us to determine critical periods when animals may become compromised, enabling humane euthanasia of animals at an early stage if it does not impact the test result, thus reducing the possibility of suffering.

Project 6	**Ex-Vivo Modelling of Cellular Pharmaco-Metabolism**	
Key Words (max. 5 words)	Cell preservation, hypothermia	
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 objectives of the project are to devise methods to extend the preservation of organ specific cells at non-freezing temperatures. This type of cell reflects the *in vivo* situation but cryopreserve poorly. Moreover due to deleterious ice crystals and the need for cryoprotectants, cells require a recovery period and show abnormal and/or reduced functionality. Factors limiting hypothermic storage are understood poorly. This project makes use of a novel procedure that maintains cells in a non-frozen state and allows the morphology of the cells to be monitored with time.	
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)?	Cell culture has both ethical and economic advantages over whole animal experiments. However, differentiated cells which maintain organ specific properties outside the body are required and their properties need validation. This type of work could reduce animal experiments, facilitate cell transplantation as an alternative to organ transplantation and may be applicable to the	

	preservation of organs.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat. 20/annum. Half of the animals to be control cell donors whilst the other half will have drug metabolising enzymes induced prior to cell isolation.
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?	No adverse effects are likely. All the induction procedures involve the mildest procedures that could be found to avoid stressing the animals. They have been tested thoroughly and produce no adverse effects. The animals are cell donors for *in vitro* work and are killed by an overdose of anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The methods being developed should enable human cells to replace animals, partly, in toxicity testing. Human cells are being used but available human tissue, used to prepare the cells, varies greatly in quality due to age, lifestyle choices and medication. This means that *in vitro* cell preservation methods can be compromised due to the quality of the starting cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To a large extent this is accomplished due to the work itself. The aim is to preserve the cells, *in vitro,* for the maximum time in a form that they behave as if the cells have been isolated freshly
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 have been chosen as cell donors. The relevant cells can be prepared reproducibly with a high yield and viability. In cell culture rat cells deviate from their counterpart *in vivo* cells more rapidly than other species. Conditions that preserve e.g. drug metabolising enzyme in rat cells should work on other species. Of particular relevance is that I have shown that the devised methods also work on human cells.

Project 7	**Detection of genotoxic substances**	
Key Words (max. 5 words)	Genotoxicity, Mutation, DNA Damage	
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)	Humans are exposed to xenobiotic materials as patients, consumers and workers. Such exposure can lead to genetic changes such as chromosome defects, point mutations and base pair deletions, which in turn can trigger tumour formation and cancer as well as other debilitating and/or life threatening conditions. In order to allow sound regulatory decisions regarding safe human exposure levels to xenobiotics, it is essential to conduct a risk assessment of the substances genotoxic activity, i.e. its ability to interact with and damage DNA. This project licence authorises the conduct of *in vivo* studies in laboratory small animal species to evaluate the hazard profile of xenobiotics in terms of genotoxicity.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The principal benefit of this project is the provision of safety data to facilitate sound regulatory decisions regarding human exposure to xenobiotics.	

project)?	
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year life of this Project Licence, it is estimated that 7,500 mice and 17,000 rats 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?	The majority of animals are expected to have mild to moderate adverse effects such as slight weight loss or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more significant adverse effects. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. All animals will be humanely euthanised at the end of a study; investigations may include sampling of various organs and tissues followed by microscopy, cytometry or *in vitro* manipulations to evaluate potential changes.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	DNA damage can occur by a diverse array of different mechanisms and at present there is no single test (either *in vitro* or *in vivo*) that is capable of detecting possible mechanisms of DNA damage. Consequently a selection of complementary *in vitro* and *in vivo* tests is required in order to determine genetic safety. The *in vivo* tests enable assessment of the effect of whole body parameters such as absorption, distribution, metabolism and excretion, which may modify the DNA damaging effects of a chemical.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Regulatory guidelines specify the minimum number of animals that should be used for the majority of tests and the numbers used are commensurate with these requirements. The studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives. Statistical input is sought, where appropriate, to strengthen the overall

	scientific quality and relevance of studies. Wherever practicable, the combination of genotoxicity endpoints and/or incorporation of these endpoints into general toxicology studies is considered, to reduce overall animal usage.
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.	Species choice and use of specific animal models is determined by the need to generate regulatorily-acceptable data. Where a choice of species is possible, the most biologically appropriate species is selected. 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 8	**In vivo DMPK/ ADME profiling and screening in rodent models**	
Key Words (max. 5 words)	metabolism, absorption, distribution, excretion, pharmacokinetics	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project licence will enable much needed research that determines the Drug Metabolism and Pharmacokinetics (DMPK), absorption, distribution, metabolism and excretion (ADME) and efficacy properties of candidate medicines and, as a consequence, aid decisions on the progression or rejection of potential new medicines in early research programmes.	
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 progression of potential new medicines can take many years. Offering clients this service will generate robust data which will allow them to take prompt actions and progress safe and efficacious compounds using scientific rationale. Advancing science in a timely manner will not only benefit future generations of the human population through the development of new disease treatments, but may also reduce additional animal use in the future.	
What species and approximate numbers of animals do you expect to use over what period of time?	This licence will only utilise rodents (rats and mice) for achieving the required experimental data. Rats will be the preferred species of choice with approximately 6000 rats and 2500 mice being required over the 5-year period of this licence.	
In the context of what you propose to do to the animals, what are the expected adverse	It is not expected that adverse effects are encountered during these studies other than minor transient effects associated with techniques utilised to	

effects and the likely/expected level of severity? What will happen to the animals at the end?	enable dosing and sampling. Animals will be dosed, usually only once, with the candidate medicine and then blood samples taken in order to assess how the candidate medicine's concentration changes over time and whether this affects whether it is able to treat a disease and how long for after dosing. Dose concentrations will be kept as low as possible to ensure any adverse effects associated with the candidate medicine are minimised. Blood sampling methods will favour the least invasive possible to minimise any distress associated with taking blood samples. Whilst we cannot predict unforeseen circumstances, we only anticipate 'mild' to 'moderate' adverse effects and levels of severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research programmes have evolved substantially in the past decade and scientific disciplines are now heavily dependent on *in vitro* technologies. How the candidate medicine is absorbed and, distributed around the body and excreted from it are tested during the research of the candidate medicine. The mechanisms are complex and cannot be adequately investigated by current *in vitro* methods, therefore the properties of novel candidate medicines in complex biological systems can only be understood with a combination of *in vivo* models and *in vitro* approaches. Regulatory bodies that review and authorise new medicines to the market will not allow these products to progress without the data we plan to produce within this project licence. Thus, it is imperative we progress with this research in a controlled and ethical manner. Therefore, with the current state *in vitro* assays available, and regulatory authority requirements, *in vivo* testing remains a necessity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Discussions ahead of any animal use will aid optimisation of the study design. This will ensure best practice during technical and scientific delivery and promote confidence to clients and researchers that ethical methods and minimal animal use is adopted. Other criteria to address ethical considerations of reduction are as follows: • Clients will have to submit data to us (where allowed) that provides assurance that all *in vitro* and/or *in silico* studies have been carried out prior to submitting any animal orders.

	• We will initiate a 'pilot' study to offer data that may not be currently available or where there is no preliminary data to confirm safety under the required study conditions. • We will adopt statistical analysis in study design to ensure the appropriate and minimal animal numbers are used to meet the scientific objectives. We are continually researching new technologies and processes that will aid reduction in animal use. Progress within these technical and scientific approaches required during animal experiments are constantly challenged by researchers across the world and published data is regularly reviewed and considered by our team. Our team have a history of developing and implementing methods to reduce animal usage and these will be used on our studies whenever possible. Such methods include: • Microsamplling methods, whereby multiple small volume samples are taken from an individual animal, thereby reducing the number of animals required for a range of study types. • Cassette dosing which is where the formulation that is dosed to the animals contains up to four candidate medicines plus a control candidate medicine. This formulation is dosed to one animal as opposed to using one animal per candidate medicine. The machinery that analyses the samples taken from these animals can then measure and quantify each of the four candidate medicines and thus reduce the need to utilise more animals and more studies. We will ensure analysis of all samples uses highly sensitive techniques that are more consistent with those used in later stages of research, meaning the need to repeat the *in vivo* phases of a study due to inadequate or inappropriate bioanalytical methods is reduced.
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.	Early phase drug discovery uses rodents as a primary model before any further species are considered. Rodents offer the most appropriate biological anatomy to reproduce a biological/drug interaction that can be measured. Minimising welfare issues will be addressed by understanding the client requirements, collaborative decision making/planning and utilising staff with

	experience at recognising potential issues should they occur. Close working relationships will ensure the study is required and that the study design appropriately meets the objectives. The majority of studies will be short term and completed within 24 hours. Where blood samples are taken, we will use microsampling methods whenever possible to make sure the minimum sample volume is obtained using the least invasive techniques possible.

Project 9	**Metabolism of Chemicals**	
Key Words (max. 5 words)	Chemical, REACH, Residues, Metabolism	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	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 satisfy regulatory requirements for assessing the absorption, distribution, metabolism and excretion of chemical materials in animals, to which humans may be exposed, including residues of agricultural chemicals in tissues and products of animals which may contribute to human food.	
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 the project is to assist in the protection of people, and animals in the environment, who may be exposed to chemical materials, by conduct of relevant tests in animals, as described by current regulatory guidance in Europe and in other international markets. The successful conduct of tests will help bring to market various materials which may themselves be seen to be of benefit to humans, or to the environment, including for example, safer or more effective plant protection products to enable higher yield of crops, or reduced danger to workers; intermediates involved in the manufacture of safer or more effective medicines for patients. Without these	

	studies, manufacture, transport and use of such materials could not occur in the current regulatory framework. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that the following may be used: • 5000 rats • 1200 mice • <100 guinea pigs and rabbits • 50 cattle • 50 goats • <lO sheep and pigs • 300 chickens • 250 fish species (rainbow trout)
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?	There is no regulatory need for test items to cause any significant adverse effects to animals. A degree of discomfort is likely to be associated with the need for restraint or confinement in order to collect samples (typically urine and faeces) non-invasively for analysis. This may be considered to be within the mild severity band or occasionally moderate. Surgical preparation of rats for collection of samples (bile or blood) or for intravenous dosing may result in a degree of mild or possibly moderate post-surgical discomfort. Such surgery is always conducted under general anaesthesia and using post-surgical pain relief, under veterinary guidance, to minimise any possible pain or discomfort. Animals will typically be humanely killed at the conclusion of a test and tissues will commonly be collected for analysis.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternative tests to meet a regulatory need have to be approved by Regulatory Authorities as acceptable to use in the assessment of risk to humans. To date very few such methods are approved, with no non-animal alternatives for the systemic tests described in this licence. Study of skin penetration can however be conducted in vitro, following published guidelines. An estimated 90% or more of skin penetration work at the establishment is conducted using an in vitro design (using skin samples from animals and/or humans). However a small amount of in vivo work is still occasionally conducted, using rodents. However there are limit of viability of the skin samples used in the in vitro test is about 24 hours. So where absorption is slow and has to be followed over a longer time period, an in vivo study will then normally be required. In addition, where skin penetration is shown to be high in vitro, the relatively increased risk for humans will then normally require subsequent conduct of in vivo studies, in rodents, to compare results to skin penetration in vivo, and to assess metabolism and excretion of the test item. In some cases, sufficient is known about the properties of a test item to establish that in vitro work will not meet the scientific need to fully assess skin penetration and metabolism. In such cases, a triple pack of studies is typically conducted, involving human and animal in vitro skin studies, and an in vivo rat study. Some regulatory authorities, including the US EPA, may not accept the in vitro studies without accompanying in vivo data. It is hoped that this position may change during the lifetime of the licence, such that even higher than the current 90% of studies can be conducted by in vitro means only.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Published regulatory guidelines contain information on numerous study designs that will be used routinely, and these commonly have detail including

	number of animals, dose levels and length of dosing period required. By adhering to these guidelines the animal usage is kept to a minimum, while maximising potential for acceptance of test results, and avoiding the need to repeat studies. Initial short screening tests using small group sizes help to select the most promising lead compounds and appropriate dose levels for formal testing. Some tests, or some groups within a test, may be conducted in one sex only. Wherever possible studies are performed in accordance with Good Laboratory Practice (GLP), as required by guidelines for regulatory studies. This should ensure the quality of studies and acceptance of results, and remove the need to repeat studies previously performed under nonGLP conditions, at a later stage of development.
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 will be used for the vast majority of the work of the project, as acceptable to regulatory authorities. A low percentage of studies will use species of animals which contribute to human food, as these are the relevant species from which humans could be exposed to agricultural chemicals. Staff training and support documents allow identification of adverse effects if and when they develop. Prior knowledge may assist in development of appropriate animal monitoring schedules for specific test items. Additional assessments are included where such need is demonstrated by effects, as standard practice. Appropriate anaesthetic use and post-surgical pain relief, under veterinary guidance, as noted above. Careful selection of dose levels will be used to ensure that significant adverse effects can be avoided; humane endpoints are agreed and will be implemented where necessary. Veterinary assistance in review of animal health and welfare is always available.

	An active programme of continuing refinement of animal housing environments exists at the establishment, under the review of the Animal Welfare and Ethical Review Body.

Project 10	**Development of Veterinary Pharmaceuticals**	
Key Words (max. 5 words)	Veterinary, Safety, Residues, Efficacy	
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
	✓	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 conduct relevant studies during pre-clinical development of veterinary medicines, as required by regulatory authorities. The work will establish what toxic effects may be seen from use of veterinary medicines, how well such medicines work in the animals they are designed for, and what residues may be left in tissues of animals which contribute to human food (meat, milk, eggs).	
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 ultimate benefit of the project is the development of safe, new medicines for use in animals. In addition, the work helps to assure the safety of human consumers who may be exposed to residues of veterinary medicines in the products of food-producing animals. As well as assuring the safety of animals and human consumers, the successful conduct of tests will help bring to market materials which will improve the health and welfare of animals in which they are used.	

	Without these studies, progression of new medicines for animals could not occur safely, legally or ethically. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that the following may be used: • 500 rats or mice • 100 rabbits • 100 dogs • 50 horses • 750 cattle, sheep, pigs or goats • 1000 chickens or other poultry
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 toxicity and safety studies is to find out the relationship between the amount of the new medicine given to the animals and any effects seen. The kind of effects seen in animals might include mild or occasionally transient moderate effects, including decreased activity or food consumption. There are no severe protocols in the licence, and severe effects are not expected. Studies to assess blood levels of medicines or residues of medicines in tissues, milk or eggs are not expected to cause any abnormalities in animals. Studies to assess if veterinary medicines work against the diseases they are intended to, may require some mild disease effects in animals, such as diarrhoea. Animals will typically be humanely killed at the conclusion of a test to allow examination of body tissues to be undertaken. On occasion, animals can be re-used in a subsequent study, or released from the site, under veterinary guidance for re-homing.
Application of the 3Rs	
1. Replacement State why you need to use	Many non-animal alternatives are used in early stage discovery work for new veterinary medicines, before the stage of regulatory testing described in this

animals and why you cannot use non-animal alternatives	project. However the purposes of this project cannot be achieved using non-animal methods acceptable to regulatory authorities in Europe and elsewhere, who are responsible for agreeing that new veterinary medicines can be safely marketed for animals. The guidance from government regulators on meeting the stated purposes of the project, almost without exception, states the need to gather data using animals. In the very limited cases where the potential to use non-animal alternatives is suggested, then it will be explored, and such alternatives used, in agreement with the regulatory authorities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Relevant government guidelines containing information on study designs will be used where available. Where there is no definitive guidance on numbers of animals, we will use experience of related programmes, taking account of the need to use sufficient animals for studies to provide robust results, without excess. For example, initial short screening tests using small group sizes help to select the most promising lead compounds and appropriate dose levels for formal testing. Wherever possible studies are performed in accordance with Good Laboratory Practice (GLP), as required by guidelines for regulatory studies. This should ensure the quality of studies and acceptance of results, and remove the need to repeat studies previously performed under non-GLP conditions, at a later stage of development.
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	Most animals used in the project will be those species for which the veterinary medicines are being developed. This is to ensure that the medicines are safe for use in the general population of animals, and also to ensure the safety of humans who could be exposed to the residues of veterinary medicines in the food we eat. Some laboratory animals, mostly rats, are used in the project as an initial assessment of toxicity of veterinary medicines. Dogs and horses

(harms) to the animals.	may be used where they are the species for which the veterinary medicines are being developed, therefore they are the only suitable species for the work. Various methods are commonly used to minimise potential harms due to test items. For example small groups of animals may be used initially, and of a shorter duration, to carefully select appropriate dose levels of test items for larger scale studies, and to identify any relevant end-points for future studies. Similarly, in the very occasional studies where a mild parasitic infection may be required of food-producing animals, published information and guidance is used to select appropriate infection levels which in most cases will cause little or no adverse effect for the animals. Wherever possible, the animals are maintained in enriched, comfortable, safe surroundings during the tests. Sometimes housing must be changed, for example, to allow collection of urine samples. Any confinement or restraint is restricted to the minimum required, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB). Dose volumes and blood sampling volumes are similarly controlled by the AWERB. Animals are trained wherever possible to avoid stress and are cared for by dedicated staff who are familiar with their husbandry needs. The institute has won awards for its husbandry and care of animals.

Project 11	**Pharmacokinetics of Pharmaceuticals**	
Key Words (max. 5 words)	Pharmaceutical, Absorption, Distribution, Metabolism, Excretion	
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
	✓	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 conduct relevant studies during drug development in the field of pharmacokinetics (absorption, distribution, metabolism and excretion) of materials in animals, to enable approval of suitable materials to be used in human volunteers and patients.	
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 successful conduct of tests will help bring to market those materials which are safe, and ultimately shown to be effective in the treatment, prevention or diagnosis of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur safely. The conduct of animal tests before human subjects can be exposed to new medicines is a legal and ethical requirement. Conduct of such studies also enables the rejection of those test items seen to be unsuitable during development. The ultimate benefit of the project is then the	

	development of safer and more effective medicines. The contribution this project makes to that aim is to protect healthy volunteers and patients who may be given potential new medicines as part of the clinical development of new treatments. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that up to the following numbers may be used: • 5000 rodents (mostly rats or mice, possibly small numbers of hamsters or guinea pigs) • 50 rabbits • 450 dogs • 450 macaques • 50 pigs • 20 marmosets
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 work in the project is designed to assess the pharmacokinetics of test items given to animals (absorption, distribution in the body, metabolism within the body and excretion from the body, or ADME). There is no need for test items to cause any significant adverse effects to animals. A degree of discomfort is likely to be associated with some of the regulated procedures required: the need for temporary single housing and restraint or confinement in order to collect samples (typically urine and faeces) non-invasively for analysis; surgical preparation of animals for collection of samples (bile or blood) or for intravenous dosing. Discomfort may be considered to be within the mild severity band or occasionally moderate for a short period. Occasionally, tumours may be implanted in animals in order to ensure that a test medicine does actually get distributed to the tumour cells while in the living animal, thereby enabling its effect. Some degree of mild, or possibly moderate discomfort may be seen, but are limited by applying end-points as advised in

	current guidance on use of animals in cancer research. Animals will commonly be humanely killed at the conclusion of a test to allow examination of body tissues to be undertaken. Animals which have not been significantly or permanently harmed by conduct of a study, may be re-used in a subsequent study, under veterinary guidance.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of the project is to find out how a whole living animal absorbs, changes and excretes potential new medicines. Methods which use non-animal replacements, or tissues or cells collected from animals, are routinely used in drug development programmes. However there is no non-animal alternative that can replace the whole living animal. This is accepted by regulations governing the licensing of new medicines.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Relevant government guidelines containing information on study designs will be used where available. Where there is no definitive guidance on numbers of animals, we will use experience of related programmes, taking account of the need to use sufficient animals for studies to provide robust results, without excess. Where possible and relevant to the particular species, animals are used sequentially in the different phases of a multi-phase study, thereby reducing animal use and resulting in more robust data as there is no inter-animal variability of results in the different phases. Where studies do not require post mortem analysis or collection of tissues, there is potential to re-use those animals which have not been harmed by conduct of procedures, under veterinary guidance and control.

	Studies are performed in accordance with the principles of Good Laboratory Practice (GLP), This requires the highest standards of staff training, study planning and data recording and storage. This minimises any risk that studies are unsuccessful and have to be repeated.
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.	Regulatory studies of ADME will use the same species of animals as used in the toxicology studies. Therefore the majority of animals used in the project will be rodents (usually rats). Other rodent and non-rodent species used routinely in toxicology studies will also be used, including guinea pigs, rabbits, dogs, pigs and non-human primates. Species with special protection are used where they are the only suitable species, or in case of dogs, where there are no other practicable species available. Non-human primates are only used in programs in the development or testing of drugs for the treatment, diagnosis or prevention of life-threatening conditions in humans. Wherever possible, the animals are maintained in enriched, comfortable, safe surroundings during the tests. Sometimes housing must be changed, for example, to allow collection of urine samples. Any confinement or restraint is restricted to the minimum required, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB). Dose volumes and blood sampling volumes are similarly controlled by the AWERB. Animals are trained wherever possible to avoid stress and are cared for by dedicated staff who are familiar with their husbandry needs. The institute has won awards for its husbandry and care of animals.

Project 12	**Novel delivery systems for pharmaceutical agents**	
Key Words (max. 5 words)	Pharmacokinetics, bioavailability, tolerability	
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 overall aim of this project is to develop novel delivery systems for pharmaceutical agents (i.e. to develop pharmaceutical formulations for treatment of specific disease indications). This will be achieved by determining the pharmacokinetic fate, bioavailability and tolerability of pharmaceutical agents in healthy animals. The formulations will be given by conventional routes of administration (in the first instance orally and into the eye). A critical part of this work is to obtain an understanding of the relationship between the dose of the pharmaceutical agent administered and the amount in blood or other tissues. The pharmaceutical agents investigated will already be licensed for human use and therefore there will be considerable existing knowledge on their safety in animals and/or humans. The novel aspect of this work is the unique composition of the pharmaceutical formulation which will be investigated as a means of improving the delivery of the pharmaceutic agent when given either by the conventional clinical route or by an alternate route.	

	The data produced in this project will be used to decide whether enough of the pharmaceutical agent can safely be delivered to treat the disease indication as a basis for developing novel pharmaceutical products which are more efficacious, better tolerated, and/or safer than current treatments. The information obtained will also be used to support applications to regulatory authorities and ethical review bodies to support human clinical trials or other investigations (e.g. toxicology studies conducted at CROs).
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 overarching benefit of this project is the potential to improve the delivery of existing pharmaceutical agent(s) which would be expected to translate into increased patient acceptability and adherence to treatment, improved efficacy and/or safety in the clinical setting when compared to current medications thereby providing an opportunity to develop products that serve unmet medical needs.
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that a maximum of 588 rats and 252 rabbits may be used over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In the majority of animals, only mild transient adverse effects are expected as a result of the procedures that are carried out (e.g. slight discomfort after dosing and sampling procedures). Around 10% of rats and rabbits that are dosed into the eye (equating to approximately 8% of the total animals used) could experience adverse effects of moderate severity (e.g. discomfort and damage to the eye). These animals will be given appropriate veterinary and husbandry treatment. Any which fail to respond promptly and effectively such that a 'normal/good' quality of life is not restored within a reasonable time will be humanely killed (a 'normal/good' quality of life is defined as one where the animal 'is able to live a standard, regular, good life without pain, suffering, distress or lasting harm'). At the end of the procedure, animals will be humanely killed and where appropriate tissue samples will be collected for histological examination as a

	quantitative assessment of tolerability.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Assessment of pharmacokinetics, bioavailability and tolerability is crucial to support the regulatory approval of potential new medicines for use in human clinical trials. Current non-animal alternatives cannot mimic the full range of events and interactions which occur within humans and animals, especially in the area of pharmacokinetics. Therefore, the project aims/objectives cannot be met without use of animals. Non-animal alternatives will be used however (e.g. formulation development and analytical studies to ensure formulations are viable, stable and suitable for purpose, cell culture and in silico techniques) to limit the number of formulations which are tested in animals. The information from such non-animal studies will inform the project licence holder of the formulations most likely to provide improved drug delivery by the chosen route of administration.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals to be used will be examined on a study-by-study basis in order to achieve scientific outcomes with minimum use of animals. Good experimental design methodologies will be applied to ensure that the minimum number of animal are used. As detailed above, studies of crossover design require use of fewer animals and will be used whenever possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Animal testing is obligatory during the development of new pharmaceutical products to ensure that they are effective and safe prior to testing in human clinical trials. Therefore, outcomes in animals need to reliably predictive of those in humans. Mammals are the most appropriate choice as physiology and metabolism is similar to that in humans; this is in contrast to observations in animals having a lower degree of neurophysiological sensitivity. The mammals (rats and rabbits) used in this project are the most appropriate choice as physiology and metabolism is similar to that in humans and these are accepted models of drug delivery. The methods that will be used are consistent with those used in human

	trials the most notable differences being the use of anaesthetic agents in animals and terminal procedures. A local anaesthetic agent will be administered to animals prior to blood sampling during studies to prevent pain from needlesticks (a temporary, indwelling, cannula may also be used to reduce the number of needlesticks). General and local anaesthetic agents will be used prior to ocular dosing and analgesics after dosing to prevent pain; local antibiotic will also be given to prevent eye infection. Use of anaesthetic agents, analgesics, antibiotics and any other veterinary treatment will be under veterinary supervision.

Project 13	**Safety Pharmacology**	
Key Words (max. 5 words)	safety, pharmacology, telemetry, regulatory	
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
	✓	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 fulfil regulatory requirements for new human drug submissions in the field of safety pharmacology To identify undesirable properties of test item that may have relevance for their human safety To investigate and further evaluate adverse effects observed and/or suspected	
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 the project is to assist in the assurance of human safety by conduct of relevant tests in animals, as described by current regulatory guidance in Europe and in other international markets. The successful conduct of tests will help bring to market test items which are safe, and shown to be effective in the treatment or prevention of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework.	

	Early cessation of programmes of work with unsuitable test items will reduce overall animal use in a drug development programme. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that the following may be used: • 1500 rats or mice • 60 dogs • 15 pigs • 20 macaques
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 regulatory need is to demonstrate any relationship between the dose level of a test item and any adverse effect observed, include its onset and duration post-dosing. In order to do this, it is expected that one or more dose levels of test items will results in measurable adverse effects for animals such as increased breathing, heart rate, blood pressure or general activity. Potentially a toxic effect may be seen as shown by, for example, decreased activity or food consumption. Non-rodents may be surgically prepared with devices to enable collection of data on measures such as heart rate and blood pressure without any form of capture or restraint. Data collected in this way are more representative of the animals' true condition than if restrained, but animals may experience a degree of discomfort from surgery, which minimised by appropriate veterinary care. Rodents will typically be humanely killed at the conclusion of a test. Non-rodents will typically be assessed by a veterinary surgeon for lack of significant or lasting effects, and may be re-used in multiple studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Current regulatory guidelines indicate the need for conduct of both non-animal methods and those which use animals, in this field of regulatory work.

use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Previous work has been conducted and published, to assess the statistical power of group sizes required for the various study types in the project. Control groups are commonly included to ensure that studies are robust and do not need to be repeated. In some cases, a single control group can be used for two or more test items, reducing overall animal use. Studies with non-rodents commonly use a design where each animal acts as its own control, by measuring effects in sequence, at different dose levels, and with no test item at all, in the same animal. Re-use of animals is employed in the project as a means to reduce overall animal use. This is particularly relevant where animals are surgically prepared; use of the same animal in multiple studies following initial surgical preparation reduces the total number of animals which are subject to surgery.
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 regulatory guidance on safety pharmacology establishes the important body systems which must be assessed for human safety, due to their importance in life-supporting functions. Studies are designed to assess effects on these body systems; principally the heart, lung and brain functions. Rodents can be used for much of this work, but are not appropriate for some assessments of heart function, as they do not compare well with human risk in this area. Use of non-rodents including dogs, pigs and primates does allow a better assessment of human risk, enables comparison between different test items in the same animals by re-use of the animals, and reduces overall animal use as noted above. Non-human primates are used where the particular type of test items requires such species as the best option to assess human risk,

	due to the similarity of humans to non-human primates. Staff training and support documents allow identification of adverse effects if and when they develop. Prior knowledge may assist in development of appropriate animals monitoring schedules for specific test items. Additional assessments are included where such need is demonstrated by effects, as standard practice. Careful selection and escalation of dose levels will be used to ensure that adverse effects can be identified but should not cause life-threatening toxicity. Effects may be seen as described above, but will commonly be only for a short period; persistent moderate clinical signs will result in cessation of further testing at the relevant or higher dose levels. Where surgery is conducted it is done so subject to all relevant veterinary assistance in terms of use of sterile materials, provision of appropriate anaesthetics and suitable pain relief schedules.

Project 14	**Safety Pharmacology Assessments**	
Key Words (max. 5 words)	Safety Pharmacology	
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)	Safety pharmacology studies are a regulatory requirement. Typically the Core battery and follow up studies are carried out prior to first exposure in man and as such a key benefit of this programme of work is to provide vital safety information to physicians responsible for protecting humans volunteering to participate in clinical trials. However, certain tests (those related to abuse potential) may be carried out later into the clinical development 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)?	In the case of medicinal products, achievement of the objectives of this licence enables safe development candidates to progress into clinical testing and to 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 medicines to early human studies and to patients/marketing could not occur.	

What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year life of this Project Licence, it is estimated that 6,450 mice, 13,600 rats, 1050 guinea pigs, 1000 rabbits, 500 dogs, 350 mini-pigs and 200 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 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 number 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; analgesics will be provided as required throughout the recovery phase. Safety Pharmacology studies are typically conducted following early toxicology studies and as such the dose levels can be carefully selected in order to avoid undue toxicity, but such that the regulatory guidelines are met. For seizure liability assessments convulsion is anticipated and necessary in order to fully evaluate the safety of the test agent. For these studies the frequency and duration of convulsant activity will be closely monitored and human endpoints modified to ensure that the welfare of the animal or the scientific purpose of the study are not compromised in any way. The majority of animals will be humanely killed at the end of a study to ensure that suffering cannot occur.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The regulatory authorities are obliged to protect human volunteers for clinical trial by requiring use of proven test systems and accepting use of animals

use non-animal alternatives	until an in vitro alternative has been demonstrated to be reliable and reproducible. The ICH guidance documents S7A suggests that a combination of in vitro and in vivo tests are necessary prior to phase I clinical trials. These include isolated organs and tissues, cell cultures, cellular fragments, subcellular organelles, receptors and enzymes. Such assays are used in early drug development and in subsequent studies to elucidate the mechanism of action. For evaluation of safety prior to Phase I, however, animals have to be used since they provide the integrated homeostatic environment which best predicts human safety. There are many aspects of the human body which can best be simulated in animals - for example, the blood and cardiovascular system, the lung and the gas-exchange mechanism, the brain which controls behaviour and cardio-respiratory mechanisms. According to the guidance S7B, in vitro electrophysiology studies can provide valuable information concerning the effects of a test substance on action potential duration and br cardiac ionic currents. However, only intact animal models allow investigation of the full functionality of the heart with all ion channels involved. 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. Wherever practicable, the combination of endpoints eg CNS, cardiovascular, withdrawal, respiratory within general toxicity studies is considered in order to

	reduce overall animal usage.
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.	Species choice and use of specific animal models is determined by the need to generate regulatory-acceptable data. Where possible, biologically relevant data are obtained from rodents, with dogs or non-human primates only used when data must be produced in these species and no other species will do. The majority of the tests are carried out in rodents (usually rats or mice). The species chosen for the toxicology programme would generally be used in the safety pharmacology package of core battery studies. Rodents are typically not suitable for the core battery cardiovascular studies. This is because the ionic mechanisms of the heart in adult rats and mice differ from larger species including humans. Moreover, a large background of cardiovascular data exists for the dog and primate, which are also a species of choice in majority of toxicology programmes. 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 always possible. For example, 24 hour cardiovascular recording, behavioural observations and time course assessments during repeat dose administration. In addition assessment in conscious animals is a requirement by the Regulators (e.g. S7A guidance). 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 15	**Environmental toxicology and metabolic fate**	
Key Words (max. 5 words)	Environment, safety, pollution, chemical, pharmaceutical	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	✓	Regulatory use and routine production
	✓	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		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 satisfy regulatory requirements for assessing the safety or metabolic fate in fish, and potentially amphibians, of various categories of chemical and pharmaceuticals materials, to which such animals may be exposed in the environment as a side-effect of production or use.	
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 the project is to assist in the assessment of environmental impact following the use of various pharmaceuticals, pharmaceutical intermediates, agrochemicals, veterinary medicines and chemicals which may find their way into water supplies and the environment following manufacture or use. The perceived value of individual test items may vary. However it is important to establish the environmental impact of all such materials, and such work is a requirement of current regulations governing the marketing of such materials in	

	Europe and in other international markets.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that up to about 10000 fish may be used, including the following species: rainbow trout, bluegill sunfish, zebrafish, fathead minnow, possibly also sheepshead minnow, freshwater carp, turbot. Potentially, small numbers of amphibians may be used if added by amendment, but these animals do not form part of the application.
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 technical procedures involved in the project are not anticipated to give rise to any significant harms. Any significant potential harm is likely to arise from test items used. One protocol in the licence is to assess toxicity of test items to fish, up to a maximum concentration established by regulatory authorities. Test items may prove toxic below this threshold, and in such cases, a range of adverse effects may be seen, including abnormal behaviour. On occasion, severe effects may be noted, requiring euthanasia of animals, or animals may die before euthanasia can be undertaken, although this is never desired or required. Animals will typically be humanely killed at the conclusion of a test and pathological assessment of tissues may be conducted.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where environmental risk assessment indicates that environmental effects must be assessed, Directives and Regulations governing the marketing of materials currently may require the use of both vertebrate and invertebrate species. Where materials are produced in low volumes, invertebrates only may be required.
2. Reduction Explain how you will assure the use of minimum numbers	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Regulatory guidance sets out the numbers of animals

of animals	anticipated to be used in different types of study. By adhering to these guidelines the animal usage is kept to a minimum, while maximising potential for acceptance of test results, and avoiding the need to repeat studies. Where materials are seen to be relatively non-toxic in invertebrates, a threshold approach can be used such that reduced numbers of fish can be tested in at the upper limit of toxicity only. Wherever possible studies are performed in accordance with Good Laboratory Practice (GLP), as required by guidelines for regulatory studies. This should ensure the quality of studies and acceptance of results, and remove the need to repeat 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.	The fish species used in the licence will be in compliance with relevant regulatory guidance for the tests and test item types involved, to meet the stated objective(s). The technical procedures involved in the project are non-invasive in nature, and are therefore not anticipated to give rise to any significant harms. The environment provided for the fish is closely controlled and monitored to minimise potential harms. Harm may arise from the effects of test items. Methods used to minimise this potential include the use of dose ranging studies using a small number of animals to collect sufficient data on the test item, potentially resulting in reduced animal usage and severity in a main study, as well as the need for further testing. The use of appropriate end-points by personal licensees, minimises severity for individual animals. Veterinary assistance in review of animal health and welfare is always available.

Project 16	**Pyrogen Testing**	
Key Words (max. 5 words)	Pyrogen Testing, Rabbit	
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)	This project ensures the ongoing safety of medicinal products used in the clinical care and support of seriously ill patients, on behalf of a not-for-profit organisation.	
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 provision of safe therapeutic products that are life saving or life preserving.	
What species and approximate numbers of animals do you expect to use over what period of time?	3750 rabbits 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	Animals may become distressed or agitated as a result of restraint. Slight elevation of body temperature may occur. At the end of the protocol, animals will be euthanased or transferred to other projects for re-use where this is authorised.	

happen to the animals at the end?	Severity - Mild
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The inherent variability of the raw material used to manufacture plasma products in the developing world means that, despite suitable quality assurance of processes at the time of collection and initial processing, results from in-vitro assays such as LAL and MAT cannot be consistently achieved. This finding follows many years of investigation and procedural refinement that continues to be improved. Unfortunately, for these invaluable plasma products, no suitable test with similar sensitivity or sufficient breadth of coverage for undefined contaminants is currently available as a direct replacement for the rabbit pyrogen test although attempts to validate alternative tests not using animals is ongoing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The requirement for three rabbits per group is set out in the European Pharmacopoeia Monograph. No further reductions are possible without an update of the Pharmacopoeia Monograph.
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 rabbit is the only animal described in the European Pharmacopoeia Monograph for pyrogen testing. This is a specialist facility in which the breeding and testing programs have been integrated to ensure the highest welfare standards are observed during the efficient performance of the test. Improvements in husbandry practice, including group housing, dietary enrichment and selective breeding have been identified and introduced. A very calm and constant environment has been established for the procedures to be conducted, ensuring the animals are comfortable and relaxed before, during and after the procedure.

Project 17	**TOXICOLOGICAL EVALUATION OF NOVEL THERAPEUTICS**	
Key Words (max. 5 words)	Toxicology, dose range finding, safety, side effects, carcinogenicity	
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 aim of this project is to identify any harmful toxic side effects of substances with the potential to become clinical drugs and to prevent drugs with unwanted side-effects being prescribed to patients. To do this, substances will be tested in animals and toxic effects will be evaluated. The most appropriate dose rates and administration routes, and toxic side-effects will be assessed, data will be submitted to regulatory authorities to make a decision whether drug candidates can be 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 work under the authority of this project licence will identify unwanted/severe side-effects of novel drugs so as to enable the weeding out of potentially harmful substances from further development and future use in people or animals. This project will also enable evaluated non-toxic drugs to progress onto clinical trials and to support drug marketing authority applications. Although beyond the scope of this	

	licence application, but as a direct result of this project, some potential drugs can reach marketing stage and be applied to treat many human diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	For initial toxicological drug evaluation up to 1000 mice/rats/year would be used. Some promising drugs would prompt follow-up studies in larger animals, as required by legislation, and for these purposes we would use no more than 250 rabbits and 60 pigs 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 maximum severity limit under this protocol is moderate, however we do not expect animals to experience adverse effects and thereby the expected severity for most animals would be mild or unclassified. The animals will be given the test drug, will be observed and have small blood samples taken in order to test how much drug is left in the body. To reduce stress and pain during multiple blood collection procedures, a small silicone tube (cannula) can be surgically inserted permanently into the vein. Urine/faecal samples may also be collected by placing animals into approved specifically-designed individual cages with a permeable floor. Collection of blood, urine or faeces will not result in any significant adverse effects. Minor surgical procedures, such as cannulation, require the use of anaesthetic, however any significant complications are unlikely. Whilst drug administration is expected to cause no more than transient discomfort, it is possible that some substances at high doses may cause more significant discomfort. Animals will be observed frequently during the study for changes in normal behaviour along with signs of stress such as non-socialising and body weight loss. Careful animal observations and assessment by trained experienced personnel will allow to identify drug adverse effects which cause pain and distress; such a response will be addressed by provision of necessary treatment or pain relief, or may result in the painless killing of the animal, if unresponsive to medical treatment.

	At the end of all studies the animals will be painlessly and humanely put down by the methods approved by the Home Office and UK/EU legislation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Computer modelling and laboratory studies would normally have been completed on the potential drugs proposed for testing under this project licence. However, data generated using computers or cell and tissue cultures has limited application to the whole body response to potential drugs. It is necessary to determine the behaviour of novel drugs in living systems to minimise the risks to human patients. It is also important to select the most appropriate dose rates and administration routes for maximum effect. Whole body assessments as described in this licence are therefore required by statute by regulatory authorities before prospective drugs can be administered to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The careful selection of test compounds from laboratory studies will ensure that only non-toxic potential drugs with a positive profile for efficacy will be taken forward for use in regulated procedures. The design of proposed experiments will be rigorously considered in order to ensure that a minimum of animals are used at all stages without compromising the integrity of the work. The services of the statistician will be used to decide how many animals are needed to ensure adequately robust results from these studies. To further help achieve this, study protocols will be constructed with a view to avoiding significant differences between experimental groups and between experimental and control groups by ensuring identical study conditions/environment for all animals and animals will be of the same strain and gender and of similar age and weight. If suitable, a single control group could be used for multiple studies to reduce the number of animals used. In most cases, a small test study will be performed prior to commencement of the main experiment to ensure the study design and specific protocol is suitable and to provide an indication of possible results.

	Unsuitable studies can then be abandoned or modified without the use of unnecessarily large numbers 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.	Mice and rats, as species of the lowest acceptable order, are the research species models of choice in drug discovery and development due to their size and substantial amount of literature data already available. These models have been used and validated extensively, and have provided much of our knowledge to date in toxicology studies which we plan to utilise to generate the package of high quality, robust and incisive pre-clinical data that we aim to provide. However, on occasion other species (i.e. rabbits/pigs) may provide a much better similarity to humans, such as forming similar drug by-products or exhibiting responses to a treatment that are much more reflective of humans and in such cases we will ensure the most relevant species are always used. The choice of animals are also dictated by the governmental regulatory bodies which demand that prior to first-in-man studies, toxicology data are provided in two species, rodent and a non-rodent, to ensure that the drug is suitable for human use. We will continuously monitor the literature to implement the latest animal husbandry legislation and practices. Furthermore, we will minimise animal suffering by using the most advanced technologies where possible, like non-invasive imaging, and by using appropriate anaesthetics, pain relief and infection control.

Project 18	**Toxicology of Pharmaceuticals**	
Key Words (max. 5 words)	Pharmaceutical, Toxicology, Safety, Preclinical, Nonclinical	
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
	✓	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 undertake safety assessment of potential new human pharmaceutical materials, by the conduct of tests required by regulatory authorities, to enable approval of suitable materials to be used in human volunteers and patients.	
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 successful conduct of tests will establish scientific information on the safety of potential new human medicines. This information is designed to be used to help bring to market those materials which are safe, and ultimately shown to be effective in the treatment, prevention or diagnosis of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur safely. The conduct of animal tests before human subjects can be exposed to new medicines is a legal and ethical requirement. The ultimate benefit of the project is therefore the development of safer and more effective	

	medicines. This is by identifying safe new medicines for healthy volunteers and patients and rejecting unsuitable or unsafe candidate drugs before humans would receive them. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that the following may be used: • 50000 rats, mice or hamsters • 2000 rabbits • 3500 dogs • 500 pigs • 2800 macaques • 100 marmosets
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 toxicity studies is to find out the relationship between the amount of the new medicine given to the animals and any effects seen. Medicines are given by the same route that humans would receive them, samples are taken for analysis; animals may be confined or restrained during the conduct of the studies. A small number of animals undergo surgical procedures to enable the dosing or sampling. The kind of effects seen in animals might include decreased activity or food consumption. Occasionally more severe effects may be seen in some animals. In this case action will be taken to alleviate suffering such as stopping the treatment, or humanely killing the animal. Rarely, sudden severe effects may be noted. In this case the affected animals are humanely killed without delay. Animals will typically be humanely killed at the conclusion of a test to allow examination of body tissues to be undertaken. On occasion, animals which have not been significantly or permanently harmed by conduct of a study may be re-used in a subsequent study, under veterinary guidance. The study designs and potential effects of test items result in consideration of most of the work

	authorised by the licence as moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of the project is to find out how a whole living animal responds to a new medicine. Cell cultures, tissue slices and many other non-living systems exist and can provide information on the way drugs act and how they produce particular effects. Non-animal methods are used routinely to identify only those most promising potential new medicines for further testing in animals and humans. But there is no non-animal alternative that can replace the whole living animal, and so animal tests and then human tests remain necessary in the development of safe and effective medicines. Alternatives have been introduced for skin tests and eye tests and are used. That is why there is no skin and eye testing for irritancy included in this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Relevant government guidelines containing information on study designs will be used where available. Where there is no definitive guidance on numbers of animals, we will use experience of related programmes, taking account of the need to use sufficient animals for studies to provide robust results, without excess. Initial short screening tests using small group sizes, are often used to select the most promising lead compounds and appropriate dose levels for formal testing. Studies are performed in accordance with the principles of Good Laboratory Practice (GLP), This requires the highest standards of staff training, study planning and data recording and storage. This minimises any risk that studies are unsuccessful and have to be repeated.
3. Refinement	Rats and mice will be used for the majority of the work of the project. However, government

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.	authorities require new drugs to be tested in non-rodents as well as rodents in order to ensure as far as possible that the new medicine is safe. These non-rodent studies form a very low percentage of the overall testing. Species with special protection are used where they are the only suitable species, or in case of dogs, where there are no other practicable species available. This is because, after man, more is known about the anatomy, medicine and disease of the dog than any other animal. Pigs are sometimes used especially for skin treatments. Monkeys are used only when no other species is suitable and when the medicine is being developed for the treatment, diagnosis or prevention of debilitating or life-threatening conditions in humans, such as cancer or Parkinson's. In every case the utmost care has been taken to ensure that the animals are maintained in enriched, comfortable safe surroundings during the tests. They are trained wherever possible to avoid stress and are cared for by dedicated staff who are familiar with their husbandry needs. Any confinement or restraint is restricted to the minimum required, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB). Dose volumes and blood sampling volumes are similarly controlled by the AWERB. The institute has won awards for its husbandry and care of animals.

Project 19	**Toxicology of Chemicals**	
Key Words (max. 5 words)	Chemical, Toxicology, REACH	
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
	✓	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 satisfy regulatory requirements for assessing the safety of various categories of chemical materials, so that they may be safely manufactured, transported and used.	
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 the project is to assist in the assurance of safety of people involved in the manufacture, transport and use of human by conduct of relevant tests in animals, as described by current regulatory guidance in Europe and in other international markets. The successful conduct of tests will help bring to market various materials which may themselves be seen to be of benefit to humans, or to the environment, including for example, safer or more effective plant protection products to enable higher yield of crops, or reduced danger to workers; intermediates involved in the manufacture of safer or more effective medicines for patients. Without these studies, manufacture, transport and use of such materials could not occur in the current regulatory	

	framework. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Within the five year life of the project it is estimated that the following may be used: • 30000 rats or mice • 500 rabbits • 250 dogs
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 regulatory need is to demonstrate any relationship between the dose level of a test item and any adverse effect observed, include its onset and duration post-dosing. In order to do this, it is expected that one or more dose levels of test items will results in measurable adverse effects for animals, such as decreased activity or food consumption. Occasionally moderate severity effects may be noted in animals, reaching the limit of severity set out in the licence protocols and requiring the implementation of humane end-points. Rarely in the course of the project, severe effects may be noted. Animals will typically be humanely killed at the conclusion of a test and pathological assessment of tissues will be conducted. On occasion, animals which have not been significantly or permanently harmed by conduct of a study, may be re-used in a subsequent study, under veterinary guidance.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternative tests have to be approved by Regulatory Authorities. To date very few such methods are approved, with no non-animal alternatives for systemic toxicity available. Where *in vitro* or *ex vivo* tests have been developed to reduce or replace animal use they are implemented, or *in vivo* work may not be conducted. For example skin irritation and corrosivity testing will not be conducted by *in vivo* means under the authority of this licence.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed. Published regulatory guidelines contain information on numerous study designs that will be used routinely, and these commonly have detail including number of animals, groups, use of control and length of exposure. By adhering to these guidelines the animal usage is kept to a minimum, while maximising potential for acceptance of test results, and avoiding the need to repeat studies. Initial short screening tests using small group sizes help to select the most promising lead compounds and appropriate dose levels for formal testing. Some tests, or some groups within a test, may be conducted in one sex only. If possible, multiple compounds within the same project may be tested together with a common control group. Multiple end-points may be assessed using the same group of animals as opposed to conduct of separate studies for the same series of purposes. Wherever possible studies are performed in accordance with Good Laboratory Practice (GLP), as required by guidelines for regulatory studies. This should ensure the quality of studies and acceptance of results, and remove the need to repeat studies previously performed under non-GLP conditions, at a later stage of development.
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	Rodents will be used for the vast majority of the work of the project, as acceptable to regulatory authorities. A low percentage of studies require the use of non-rodent species in compliance with published guidelines. Staff training and support documents allow identification of adverse effects if and when they

(harms) to the animals.	develop. Prior knowledge may assist in development of appropriate animals monitoring schedules for specific test items. Additional assessments are included where such need is demonstrated by effects, as standard practice. Careful selection and escalation of dose levels will be used to ensure that adverse effects can be identified but should not cause life-threatening toxicity. Effects may be seen as described above, but will commonly be only for a short period; humane end-points are agreed and will be implemented where necessary. Veterinary assistance in review of animal health and welfare is always available. An active programme of continuing refinement of animal housing environments exists at the establishment, under the review of the Animal Welfare and Ethical Review Body.