Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 13

Projects with a primary purpose of Regulatory use/ Routine production

Project Titles and Keywords

1. **Efficacy and safety of products for the prevention and control of disease in equines**
    - efficacy, safety, vaccine, veterinary medicine, equines

2. **Supply of biological materials**

3. **Production of Biological Samples**
    - Blood; Urine; Sampling.

4. **Statutory and Endemic Avian and related Virus Diagnosis**
    - Health, poultry, virus, disease, diagnosis

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

6. **Efficacy and safety testing of vaccines**
    - Veterinary medicinal product, Marketing authorisation

7. **Production of antigen for licenced ovine chlamvdia vaccine**
    - Antigen vaccine

8. **Regulatory Testing of Biological Toxins**
    - Biological Toxin Potency, Assay Antitoxin

9. **Fish disease control: preventative treatments in relation to environmental factors**
    - Aquaculture; bacterial pathogens; control methods; probiotics; vaccines

10. **Efficacy and safety of products for the prevention and control of disease in farm animal species**
    - Efficacy, safety, vaccine, veterinary medicine, farm animals

11. **Safety and efficacy of anti-parasitics in food producing animals**
    - Efficacy, safety, anti-parasitics, farm animals

12. **Rodenticide Research And Development**
    - Rodents, Rodenticides, control, toxicity, palatability

13. **Nutrition of poultry**

14. **Development of Poultry Coccidiosis Vaccines**

- Poultry Coccidia Eimeria Vaccine

15. Testing of Chemicals on fish for use offshore

- Ecotox testing – Juvenile fish

16. Inhalation Toxicology - Chemicals

- Inhalation, Aerosols

17. Toxicology: Medical Products

- Regulatory Toxicology, Safety Assessment

18. Genetic Toxicology – Chemicals

- Mutagen, Genetic Toxicology

19. Collection of body fluids and/or tissues for in vitro use

- Body Fluids/Tissues In-Vitro Use

20. ADME and incurred tissue studies

- ADME, veterinary, livestock, incurred residues

21. Effects of noval and other compounds on GI tract

- Gastro-intestinal system, Safety, Pharmacology, Efficacy.

22. Aquatic Ecotoxicology

- Regulatory, toxicological, data, environment

23. Assessing the Risk of Environmental Contaminants to Fish

- Fish Environment Risk

<table>
<tr><td>PROJECT 1</td><td colspan="3">Efficacy and safety of products for the prevention and control of disease in equines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">efficacy, safety, vaccine, veterinary medicine, equines</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of disease in equines. Disease control products are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td colspan="3">The overall aim of the programme of work is to develop safe and effective means of controlling disease in equines. Disease and ill health caused by disease in equines continues to be a worldwide welfare concern. This problem is being exacerbated by the rising levels of resistance to</td></tr>
</table>

project)?	various products.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 700 equines over the five year duration of the project 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?	In the majority of cases the adverse effects are likely to be minimal or mild. Where equines are challenged in order to test the efficacy of a product, then the disease model will be the least severe available in order to satisfy European guidelines. In addition, the equines will be monitored frequently, with appropriate intervention when adverse effects are observed. Where at all possible, equines will be returned to the farm environment or where appropriate sent directly for humane slaughter in the same manner as any typical farm animal. Where this cannot occur (an unregistered veterinary product for example), animals will be humanely euthanased.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to get a marketing authorisation for a veterinary medicine, efficacy and safety data for that medicine must be provided to the regulator. European guidance documents stipulate that the target species of animal, in this case the equine, is used to produce this efficacy and safety data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where there is a European guidance document detailing the requirements, we will comply with these. Where there is no guidance document, we will take the advice of a statistician.
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	The animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded. Where adverse effects are observed we will intervene to ensure that severity limits are not

(harms) to the animals.	exceeded. Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent inspection by veterinary surgeons. Animal accommodation is substantially better than that recommended in the Equine Industry Welfare Guidelines Compendium for Horses, Ponies and Donkeys, and is compliant with A(SP)A codes of practice. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed and the harms to be experienced by the equines.

PROJECT 2	**Supply of biological materials**	
Key Words (max. 5 words)	Supply of biological materials	
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[3]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of the project licence is to supply biological material to researchers to enable them to perform in-vitro and diagnostic procedures in the advancement of medical science.	
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)?	There is continuing need for simpler, more humane and cost-effective programmes to support basic biomedical research projects, identify potential ethical compounds, therapies, and other pharmaceuticals for use in man and animals, and to limit the necessity to conduct alternative in-vivo experiments. Part of this need includes the provision of high-quality primate antisera, blood, and tissue. Blood or tissue products supplied under this licence may also be used to determine suitable species for testing; susceptibility or cross reactivity of Cynomolgus macaques or to develop assays which will be used to identify candidate drugs before embarking upon extended programmes of pre-clinical and clinical testing.	

	Within the research community it is accepted that purpose bred animals are the superior model when nonhuman primates are required and maintaining a healthy colony where these procedures are highly regulated helps to provide an assurance of quality to researchers and scientists.
What species and approximate numbers of animals do you expect to use over what period of time?	Purpose bred cynomolgus primates will act as donor animals and they may be used every 28 days to supply blood to a predetermined volume. They can remain as donors as long as they continue to be suitable and in good health. Approximate number of animals to be used e.g. estimate 100 animals to be used in 2500 procedures over the course of the project.
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 most animals, small blood samples will be taken from superficial veins. Animals will be trained to cooperate willingly and/or may be sedated to facilitate handling and minimise any stress caused. Animals should experience no more than mild severity and any effects from the blood sampling and sedation, such as pain and disorientation should be transient and not long lasting. At the end of their donation period they may be humanely killed, or in some cases blood and tissues may be collected under terminal anaesthesia. Whilst consideration may be given to rehoming, it is likely the tissues from animals will be required to support in vitro testing. Due to the relatively small number of purpose bred non-human primates that are available that have never been treated with research test articles, these tissues will be in high demand to supp this important ex-vivo research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to the use of blood products which can be used for in-vitro testing
2. Reduction Explain how you will assure the use of minimum numbers	The number of animals used will reflect the scientific requirement. Sample volumes taken will be the minimum required to fulfil the needs of the particular project. Animals may be re-used provided they remain healthy, subject to evaluation by a veterinary

of animals	surgeon.
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 use of donor animals to supply blood for in vitro experiments reduces the overall number of animals required in these studies. The use of blood products from primates is very specific and vital for some aspects of diagnostic and in-vitro research. Good husbandry, behavioural management and experimental techniques will reduce the stress involved. Animals will be trained to cooperate with procedures, to minimise stress caused. Sample volumes will be limited such that no long term effects are expected, and animals will be monitored regularly to ensure they remain healthy.

<table>
<tr><td>PROJECT 3</td><td>Production of Biological Samples</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Blood; Urine; Sampling.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[4]</td><td>
<table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[5]</td><td><s>Yes</s></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To obtain blood and/or urine samples for the performance of in-vitro work which may include the validation or calibration of laboratory equipment or to establish methodology.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The potential benefits associated with the attainment of the objectives are as follows:
The biological samples collected will allow in-vitro studies to be performed and the data generated used to make an assessment of the bio-availability of a drug and an indication of its pharmacological effects before going into animals.
The validation and calibration of equipment and the establishment of methodology prior to use on future regulatory and non-regulatory studies</td></tr>
</table>

	will allow data to be collected in a GLP compliant manner which is acceptable to International regulators.
What species and approximate numbers of animals do you expect to use over what period of time?	Biological samples will be taken from rats, mice and rabbits. Animal usage over a five year period will be approximately: Rats - 400 Mice - 400 Rabbits - 50
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?	Animal use will be limited to the withdrawal of blood and the collection of urine using established methods. Any pain, suffering or distress incurred will be transient only and will generally arise from the introduction of a needle, routine handling, being housed singly in a metabolism cage and the administration of an anaesthetic. Animals will be euthanized after use.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of this project licence is to produce biological samples from protected animals for the performance of *in-vitro* work which utilise blood and/or urine, or to validate and/or calibrate equipment via the use of these products. The use of alternatives is, therefore, not possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The objective of this licence is to obtain blood and or urine samples from laboratory animals for *in-vitro* studies, to establish methodology, or to validate equipment. In the majority of cases the quantity of material required will be known. It will therefore, be easy to establish how many animals will be needed for each purpose thus ensuring that the minimum number of animals capable of achieving the objectives is used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Rats, mice and rabbits are the selected species for work performed under this licence. Blood and urine samples obtained these species are often used to perform *in-vitro* studies; for

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	example, plasma stability studies. Blood samples are taken and used as a source of enzyme. It is possible to establish the rate and extent of reduction in drug concentration in plasma/blood and serum under different incubation or storage conditions. Samples can also be used to validate and/or calibrate laboratory equipment. Samples will be taken using established techniques which have been refined to the extent of causing the animals transient discomfort only. Where objectives allow, samples will be taken under terminal anaesthesia thereby, minimising pain, suffering and distress further.

PROJECT 4	**Statutory and Endemic Avian and related Virus Diagnosis**	
Key Words (max. 5 words)	Health, poultry, virus, disease, diagnosis	
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[6]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1) - To maintain the capability of the National and International Reference Laboratory for Avian Influenza and Newcastle Disease and meet statutory requirements as laid down by OIE/EU/FAO. To give a service of enhanced diagnostic testing and to supply high quality diagnostic reagents for identifying notifiable agents. 2) –To maintain the capability of the DEFRA to diagnose all poultry viral disease to provide an accurate and reliable diagnostic service for potential disease problems within the UK poultry industry . This work is covers UK disease problems and also import/export requirements (proving freedom from disease) as laid down by the export regulations. 3) – To look at any possible new and emerging	

	viral diseases problems in poultry, influenza in pigs and all suspected viral infections in reptiles.
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 an effective and reliable laboratory service for the diagnosis of viral diseases of birds, reptiles ,and amphibians and influenza virus in pigs has a number of potential benefits. In relation to outbreaks of disease in commercial poultry, the rapid detection of viral pathogens in submitted samples assists DEFRA, the private veterinarian and/or poultry company in deciding on appropriate action. This is particularly important with regard to the identification and characterisation of Newcastle disease and avian influenza viruses, highly contagious viruses which are controlled eradication policies. This licence is essential to support the International Reference Laboratory (EU,OIE and FAO) in the control of these diseases. The results provide information on the global situation regarding these two very important transboundary viruses and enable governments and industry to implement appropriate control measures to limit the spread of the disease, improve the health and welfare of animals and protect public health and food security.
What species and approximate numbers of animals do you expect to use over what period of time?	Chicken eggs 300,000 Turkey eggs 1,500 Duck eggs 1,000 Goose eggs 1,500 Pigeon eggs 50 Turkeys 570 Chickens 7,500 Ducks 50

	Geese 120 Pheasants 5 Partridges 5 Pigeons 5 Ostrich 5 The licence is demand lead by amount of diagnosis required, these figures represent maximum numbers that may be used during the five-year span of the project.
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 the animals will not experience anything other than momentary adverse effects associated with the infrequent administration of an injection (usually only once at the start of a study), or the collection of a blood sample (for example once a week). Anaesthesia is provided where appropriate to prevent any distress. So this will fall under the mild classification. In a small number of studies under this licence clinical signs associated with a viral infection may be experienced. These tests are required under international regulations OIE in characterising High Path Avian Influenza and Newcastle Disease. The severity of these clinical signs will vary from moderate and severe depending on the strain of virus that is being diagnosed Steps will be taken to prevent unnecessary distress. These include having an increased number of observations of the animals and use of clearly defined clinical score recording to assess severity and/or progression or recovery.
Application of the 3Rs	
1. Replacement State why you need to use	A complete biological system is required either for the production of biological material or to

animals and why you cannot use non-animal alternatives	meet statutory requirements for diagnosis of infection.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animal used on this licence is primarily demand lead by the diagnostic need, with the numbers of the animals used for the invivo diagnostic tests being dictated by international standard. Molecular methodology has been developed to minimise the number of times the invivo diagnostic tests have to be carried out (e.g. only for the index case of an outbreak to enable initial full virus characterisation).
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.	This is a viral diagnostic licence and the main animal model used is eggs as these are considered the lowest sentient animal than can be used. The production of antibodies and antisera, and the collection of blood cells use birds as these are dictated by the tests that these biological products will be used in. For the invitro diagnostic tests (intravenous pathogenicity index (IVPI) and Intracerebral pathogenicity index (ICPI) chicks are required under the statutory diagnostic definition of these tests. Whilst birds are observed at least twice a day and those showing neurological clinical signs such as paralysis, are killed, some birds may die between observations. This proportion varies with the pathogencity of the virus , the lower pathogenic virus are around 20% but the highly pathogenic viruses it is around 70% due to the unpredictable and quick nature of the death. Molecular methods for determining pathogenicity are being developed and may replace the statutory invivo tests used in this licence during the life time of this licence but these tests can only be fully replaced once the technique has is recognised by international legislation

PROJECT 5	**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)	☐	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[7]
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 immediately. 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?	Rabbits are used exclusively for the production of polyclonal antisera. A maximum of 2000 rabbits 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 period s of 2 weeks to six months 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. 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 weighed prior to each procedure to monitor animals 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, similarly the growth of certain bacterium such as the one causing Syphilis cannot

	be achieved with tissue culture techniques.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The current plan of work uses animals that produce larger volumes of serum and bacteria antigens due to their size and weight (purpose bred strains). Reductions of 40-50% have been achieved in the last 6 months using 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.	Rabbits have been historically used for antibody production, they were originally chosen for their ease of use (blood sampling & antigen dosing), ease of housing, 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 and maximization of yields in the Laboratory.

<table>
<tr><td>PROJECT 6</td><td colspan="3">Efficacy and safety testing of vaccines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Veterinary medicinal product, Marketing authorisation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[8]</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[9]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The purpose of this programme of work is to establish the efficacy and safety of batches of vaccine produced by BVL, in order that the products comply with marketing authorisation in line with European directive 81/852/EEC.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Vaccination can form part of a control plan to reduce the impact of disease and as such is it essential that these vaccines are commercially available to farmers.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td colspan="3">Batch testing of vaccines must utilise the target animal for that vaccine. The number of animals per batch is determined in the marketing authorisation. As such, over 5 years it is expected that no more</td></tr>
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over what period of time?	than 180 cattle and 720 ewes 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?	For potency testing, the animals will be observed and monitored throughout the study, and will be vaccinated using the recommended marketing authorisation dose regime. The animals will be blood samples before and after vaccination to establish that a suitable immune response has been mounted to the vaccine and that a suitable level of immunity has been established. Safety test animals for Mydiavac are administered with a double dose. Mild severity levels are expected for all protocols. Animals will return to their herds of origin 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 alternative	This project plan is required in order for demonstration of stated immunological effect in target species. It forms part of marketing authorisation for this veterinary medicine product.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Attention to detail in health planning and monitoring prior to animals entering the study and during the study should ensure that the chance of failure due to external factors is minimised. FAI aims to consult with the VMD and find alternatives to the safety test, thus reducing the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Marketing authorisation requires that the vaccine is tested on the target species. The protocol has been refined to ensure that potential risks to animals at each stage are minimal. Good training in handling, vaccination technique and blood sampling technique should ensure that the potential adverse effects are maintained at the minimal low level. The handling area for cattle is of a curved design to allow good animal flow and minimal stress during routine handling.

PROJECT 7	**Production of antigen for licenced ovine chlamvdia vaccine**	
Key Words (max. 5 words)	Antigen vaccine	
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[10]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Production of Antigen for vaccine manufacture The process will allow for the Manufacture of chlamydophila abortus (formerly Chlamydia psitacci) antigen for inclusion in a vaccine for the prevention of Enzootic Abortion in Ewes. The vaccine is also to be manufactured by the licence holder.	
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 will allow for the production of an antigen to manufacture a licenced veterinary vaccine for the prevention of chlamydophila based abortion in Sheep. This will reduce the burden of disease in susceptible animals. C. abortus continues to be the most commonly diagnosed infectious cause of abortion in sheep (and also causes abortion in goats and cattle). In 2012, C. abortus was identified as the cause of abortion in 539 (34.2%) of sheep and goat submissions in which a diagnosis was reached. The equivalent figures for 2011 were 451 (40%) diagnoses from relevant sheep and goat submissions. Each confirmed case generally represents an outbreak in the source flock or herd and the total number of animals affected is therefore considerably	

	higher. What species and approximate numbers of animals do you expect to use over what period of time? Between 20-50% of abortions in sheep are thought to be due to C. abortus and about I million lambs are aborted or stillborn each year.
What species and approximate numbers of animals do you expect to use over what period of time?	Embryonated hens eggs 40,000 per 4 month manufacturing campaign with manufacturing campaigns running approximately every 18 to 24 months.
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 antigen for the vaccine is grown in the eggs as it cannot be cultured in other systems. The procedures result in a mild effect on the eggs as the multiplication of the chlamydial culture within the egg causes the embryo to become non viable prior to a sentient stage of development. The harvest point is determined by the egg becoming non viable as this represents the peak level of chlamydia within the egg.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The chlamydophila strain required for the vaccine cannot be grown in sufficiently high numbers to produce the vaccine without being cultured in an in vivo system i.e. Embryonated hens eggs. Chlamydophila abortus will not reproduce to sufficient numbers in tissue culture. Even in susceptible cell lines they will only infect the cells and not multiply.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Extensive refinement of the manufacturing method has resulted in a very high yield of antigen per egg which has reduced the number required by 200% compared to the original methods used in this manufacturing process. Additionally manufacturing campaigns will be sized to meet the known demand for the product. An ongoing process optimisation process will also be carried out to ensure the maximum yield of product per egg is maintained.
3. Refinement Explain the choice of species	The Embryonated egg production described here is the only viable method for production of the antigen

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.	and the significant levels of process refinement that have been applied to this method mean it represents the least number of animals that can be utilised to meet the goals of the project. The incubation conditions of the eggs are monitored on daily basis to ensure that the required parameters are maintained. During site shutdown hours the incubators that house the eggs are connected to a monitored alarm system which has a 24hr call out to a responsible person therefore any deterioration in the housing conditions will be swiftly detected. Eggs are candled regularly throughout the protocol to ensure that no adverse events outside the scope of the protocol have occurred. The protocol has a mild endpoint as the process results in the majority of the eggs becoming non viable prior to a sentient point in the embryo development. Viability is monitored by regular candling of the eggs from the expected point of effect onwards. Any eggs that are indicted as approaching or having reached a non viable state are humanely killed via a schedule 1 method.

PROJECT 8	**Regulatory Testing of Biological Toxins**	
Key Words (max. 5 words)	Biological Toxin Potency Assay Antitoxin	
Expected duration of the project (yrs)	5 years **+** (ongoing)	
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[11]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To undertake testing procedures to ensure the safety, efficacy and overall quality of botulinum toxins and associated proteins used for medicinal products in accordance to registered marketing authorizations held with national and international regulators and in accordance with Good Manufacturing Practice To provide testing services to assist with product development and clinical trials associated with botulinum toxins and associated proteins. To provide testing services to assist with pharmacovigilance of drug products associated with botulinum toxins and associated proteins in accordance to registered marketing authorizations held with national and international regulators and in accordance with Good Manufacturing Practice.	
What are the potential benefits likely to derive from this project (how science could be	The work detailed in this project will allow the continued safe development, production and use of botulinum toxins and their derived products for the	

advanced or humans or animals could benefit from the project)?	treatment of a wide range of medical conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Potency Assay for Botulinum Toxin: 500,000 mice Botulinum Toxin Antibody Assays: 10,000 mice Neutralisation Assay for botulinum Toxins: 10,000 mice
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Occasionally mis-injection into the lumen of the intestine could potentially cause peritonitis and mis-injecting into subcutaneous tissue can cause abscesses although the latter has never been seen in practice. Occasional injection into the bladder can occur. Careful injection by experienced technicians reduces these risks and any animal suspected of being mis-injected will be killed by a schedule 1 method. All animals except possibly those in the very low dose groups will show typical signs of the toxins to some degree; this includes difficulty with breathing (wasp waisting, deep gasping or abdominal breathing), cyanosis, ataxia, lethargy, ruffled coats, an inability to move and some limb paralysis. Some animals will recover from these signs over the course of the tests so all need to be kept alive until they are showing severe clinical signs of toxicity at which point they are killed by a schedule 1 method. Despite frequent observations (hourly) some animals may die due to the potential rapid onset of symptoms.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mode of action of toxins is complex; this cannot be fully replicated in cell culture or other in-vitro techniques and currently requires an animal model. For example the neurotoxins are proteins that all have similar molecular structure and molecular weights. They are usually associated with non-toxic protein both naturally and in- vitro. They have a di-chain structure consisting of a light chain which is the toxic portion of the molecule and the heavy chain which is responsible for targeting the cholinergic neurones. These neurotoxins act presynaptically by blocking the release of the neurotransmitter, acetyicholine, at the

	neuromuscular junction. With the exception of one botulinum toxin (Botox, Allergan) all products currently released in the world require a mouse potency assay for batch release. Botox has a toxin specific cell culture assay which is not currently validated for use on any other manufacturers toxin, even those of the same serotype.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The animal numbers required for the potency assay has been reduced as experience has been gained at closely targeting the expected values. Careful design of the assays using a geometric progression of dilutions that results in a symmetric design about the known estimated potency ensures a robust assay with maximum precision from the number of mice used in the assay designed to be appropriate to meet the regulatory requirements to safely control the production and release of the product. The number of animals currently required per annum is based on the test history of theses assays at this facility. The majority of samples received for potency assay are determined by the Routine Quality Control (QC) Assay.
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.	Historically the majority of the work undertaken on biological toxins has been with mice as the animal model being the lowest neurophysiological model considered appropriate. The mouse lethality assay for the biological toxin test requires death as an end point; however, suffering can be reduced by killing, using a Schedule 1 method, any animals that it is predicted will die during the course of the test. Mice are observed at regular and frequent intervals, those showing severe symptoms will be killed.

PROJECT 9	**Fish disease control: preventative treatments in relation to environmental factors**	
Key Words (max. 5 words)	aquaculture; bacterial pathogens; control methods; probiotics; vaccines	
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[12]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project will maintain availability of existing fish vaccines for release to fish farms and aims to develop new disease treatments and control methods for important fish diseases. Existing vaccines have to be tested under controlled conditions to ensure that they work as advertised and that defective vaccine batches are not released for sale. As new diseases and strains of existing diseases arise, it is necessary to evaluate the performance of existing vaccines and to develop new control measures, There is also a need to reduce and replace the use of antibiotics in aquaculture, to avoid issues of antibiotic resistance in human pathogens.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Ongoing work shows the potential for probiotics, feed additives and refined vaccines for the control of fish diseases without the need for antibiotics. This is likely to increase the potential to control disease in aquaculture, so helping enable the required increase of aquaculture to meet global food needs, currently critically constrained by disease. The continued	

project)?	supply of effective fish vaccines also underpins security of an important food supply across Europe. Vaccines and other new methods will alleviate suffering in fish caused by development of disease pathology. The project will advance science by identifying new ways of using existing treatments, and by the development of new prevention and / or treatment methods. it will also help to identify mechanisms of action of treatments and basic biology of pathogens, again helping to refine new control methods. People will benefit as a result of secure fish supplies from aquaculture through reduced losses to disease. Fish will benefit through improved welfare on farms as a result of reduction in pathogenic diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Numbers of fish used are likely to be in the order of 10000 rainbow trout, 200 Atlantic salmon, 1000 zebrafish and 100 flounder/plaice 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?	Adverse effects centre around the development of disease symptoms in control, unvaccinated fish, and in fish where a vaccine or treatment has been ineffective. This might include ulceration, internal and external haemorrhaging, osmoregulatory imbalance, respiratory distress and death. These effects would be severe, but would be exactly similar to the effects seen in a fish farm lacking effective vaccine, where several hundred thousand fish could be affected at once. These effects are minimised by regular monitoring of fish during investigations, and euthanasia before full-blown symptoms or death due to disease occur.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Vaccine testing requires whole animal studies as this is specified by the regulatory authorities (e.g. VMD) and is the only way to investigate the complex host-disease interface where the vaccine must operate in practice. Whole animal studies relating to environmental factors are also needed to integrate the effects indicated from cell and isolated organ studies (which are used whenever possible, and for initial studies) into the context of the whole animal system.
2. Reduction Explain how you will assure	Fish numbers will be minimised by using established methods of experimental design (power analysis), and by initially doing small scale pilot experiments to

the use of minimum numbers of animals	see how obvious the effects might be (bigger effects smaller numbers of fish required). Assessment of existing fish vaccines is regulated by external authorities who specify sample sizes.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	These species are the most relevant for assessment of fish vaccines, disease controls and environmental effects in freshwater and brackish environments. The species (rainbow trout) for vaccine trials are prescribed in the appropriate regulatory documents and so cannot be varied. They are also the most refined model for vaccine development and research, since they are the longest standing species for which vaccines were available, and they are the best researched cold-water fish species. Zebrafish are the best described warm-water species, and appropriate equipment and facilities for research on them is more advanced than for any other warm-water species. Flounders and plaice are the best researched flatfish in the NE Atlantic area, whilst flounders also have the best developed techniques for example for gill perfusion, of any fish species. Welfare costs will be minimised by using minimum numbers of fish commensurate with statistical validity and/or regulatory requirements, and using cell and organ harvest rather than whole animal studies where possible.

<table>
<tr><td>PROJECT 10</td><td>Efficacy and safety of products for the prevention and control of disease in farm animal species</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>efficacy, safety, vaccine, veterinary medicine, farm animals</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of disease in farm animals. Disease control products are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>The overall aim of the programme of work is to develop safe and effective means of controlling disease in farm animal species. Disease and ill health caused by disease in farm animal species continues to be a worldwide welfare concern. This</td></tr>
</table>

project)?	problem is being exacerbated by the rising levels of resistance to various products.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle 1400 Sheep 700 Goats 550 Pigs 1400 Chicken 12450 Turkey 1450 Duck 400
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 cases the adverse effects are likely to be minimal or mild. Where animals are challenged in order to test the efficacy of a product, then the disease model will be the least severe available in order to satisfy European guidelines. In addition, the animals will be monitored frequently, with appropriate intervention when adverse effects are observed. Where at all possible, animals will be returned to the farm environment or where appropriate sent directly for humane slaughter in the same manner as any typical farm animal. Where this cannot occur (an unregistered veterinary product for example), animals will be humanely euthanased.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to get a marketing authorisation for a veterinary medicine, efficacy and safety data for that medicine must be provided to the regulator. European guidance documents stipulate that the target species of animal is used to produce this efficacy and safety data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where there is a European guidance document detailing the requirements, we will comply with these. Where there is no guidance document, we will take the advice of a statistician.

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 animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded. Where severity limits might be exceeded, we will intervene to treat the animal. Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent inspection by veterinary surgeons. Animal accommodation is substantially better than Defra code of recommendations for the welfare of livestock and is compliant with A(SP)A codes of practice. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed

<table>
<tr><td>PROJECT 11</td><td colspan="3">Safety and efficacy of anti-parasitics in food producing animals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">efficacy, safety, anti-parasitics, farm animals</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The overall aim of the programme of work is to provide efficacy and safety data for products for the prevention and control of parasites in farm animal species. Veterinary anti-parasitics are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td colspan="3">The overall aim of the programme of work is to develop safe and effective means of controlling parasites in farmed animal species. Disease and ill health caused by parasites amongst farm animal species continues to be a worldwide welfare concern. This problem is being exacerbated by the</td></tr>
</table>

project)?	rising levels of resistance to various anti-parasitics.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle 2000 Sheep 1300 Goats 700 Pigs 1900 Poultry 3200
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 cases the adverse effects are likely to be minimal or mild. Where animals are challenged with a disease in order to test the efficacy of a medicine, then the disease model will be the least severe available in order to satisfy European guidelines. In addition, the animals will be monitored frequently, with appropriate intervention when adverse effects are observed. Where at all possible, animals will be returned to farm following veterinary certification. Where this cannot occur (an unregistered veterinary product for example), animals will be humanely euthanased.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to get a marketing authorisation for an antiparasitic, efficacy and safety data for that antiparasitic must be provided to the regulator. European guidance documents stipulate that the target species of animal is used to produce this efficacy and safety data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where there is a European guidance document detailing the requirements, we will comply with these. Where there is no guidance document, we will take the advice of a statistician.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded.

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Where adverse effects are observed we will intervene to ensure that severity limits are not exceeded. Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent inspection by veterinary surgeons. Animal accommodation is substantially better than Defra code of recommendations for the welfare of livestock and is compliant with A(SP)A codes of practice. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed and the harms to be experienced by the animals.

<table>
<tr><td>PROJECT 12</td><td>Rodenticide Research And Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Rodents, Rodenticides, control, toxicity, palatability</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[18]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim is to undertake research and development and generate efficacy data on rodenticidal baits/devices for the control of pest species of rodents (rats, mice and non UK voles). Existing rodent control relies heavily on anticoagulant rodenticide baits. However, important concerns for the long-term viability of these techniques make the search for new and improved methods a matter of high priority. Also, resistance to anticoagulants in Norway rats and house mice is a significant problem in many countries and therefore, any novel rodenticides discovered would have significant global benefits. Behavioural problems occurs when rodents (especially urban mice) show an avoidance of bait and control accessories including bait trays and bait boxes and traps. There is no simple solution to this but offering a wide-range of bait</td></tr>
</table>

	bases helps. To improve safety to non-target species baits that can be secured and do not spill and but are still palatable, effective, and weather tolerant need to be developed.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Research into improved methods of rodent control and rodenticide development, some of which necessitates animal testing, is justifiable at three levels. Firstly, the basic requirement to control rodents because of their pest status. Secondly, that existing control methods have a variety of difficulties and failings that make improved methods highly desirable and thirdly, to fulfil regulatory requirements. Novel techniques of rodent control are justifiable as current methods can be significantly improved.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, mice and voles. In the laboratory approximately 100 voles a year and between 1,500 and 2,500 rats and mice a year depending on the success of the research. In the field, that is actual infestations, between 100 and 2,000 rats and mice per year.
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?	Overall death is expected in approximately 50% of animals used. However, it is expected that 80% of all animals recorded as dead will have been culled according to humane endpoints by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rodenticides are designed to be mammalian toxicants and are thus a unique group of biocides/pesticides. The ultimate test of efficacy is lethality and this must be assessed against the target organism. It follows, therefore, that animals cannot be replaced by experimental models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1. before the commencement of animal studies, the product must be deemed 'fit for purpose'. 2. Appropriate *in-vitro* work, will be undertaken as an integral part of any study 3. using a logical sequence of *in-vivo* studies.

	when the end result is uncertain, using a group of only two animals for the initial study 4. never dosing more than two groups at any one time. adopting a step-wise approach to further doses 5. for devices, utilising 1 animal at a time with the results reviewed before proceeding with the next animal. 6. using 5 and not 10 animals per group whenever possible provided that this complies with regulatory guidelines and provides appropriate statistical accuracy.
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.	1. Humane end-points are stringently employed. Any animal exhibiting clinical signs of a type and severity which may be expected to effect death is killed, by a Schedule 1 method. 80 per cent of all animals recorded as dead will be killed according to this criterion. 2. Also, throughout the work every effort is made to develop experimental methodology to use non-lethal end-points, examples of such refinement are: a. using non-lethal end-points b. using 'blank' baits, *i.e*. containing no rodenticide, whenever possible. These have no adverse effects on the rodents. c. if only bait palatability data, *i.e*. no mortality data, is required, then animals can be humanely killed, by a Schedule 1 method, before clinical signs of toxicity are expected. d. protocols allow starvation periods of up to 12 hours, for the vast majority of procedures, the starvation period is only 6 hours. e. lethal toxicity study never dosing more than 2 groups at any one time and evaluating the results before adopting a step-wise approach to further doses. f. no wild animal will be used unless there is no alternative. g . any device (e.g. traps) must first pass an *in-vitro* assessment before using animals under terminal general anaesthesia. Positive results are required before testing on conscious animals. Studies on conscious animals are undertaken on 1 animal at a time with a review before proceeding to the next animal.

<table>
<tr><td>PROJECT 13</td><td>Nutrition of poultry</td></tr>
<tr><td>Key Words (max. 5 words)</td><td></td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[19]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[20]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objectives of this project are:

1. To determine the efficiency of utilisation of feedstuffs, including unconventional feedstuffs, by poultry species
2. To elucidate the effect of the use of feed additives in improving the utilisation of feedstuffs by poultry species
3. To examine the effect of different dietary interventions on growth, productivity and nutrient utilisation by poultry species
4. To understand the various factors that cause ill-health in foot and hocks of poultry species and dietary interventions to prevent such
5. To establish proper procedures for determining efficiency of nutrient utilisation by poultry, and
6. To understand the interactions between nutrition and poultry health, with particular emphasis on gut health, and ascertain how</td></tr>
</table>

	different dietary interventions influence ability of poultry species to resist infection
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)?	Some of the potential benefits from the project are: 1. Understanding of variability, and causes of such, in feedstuffs (conventional and unconventional) with the objective of reducing competition between man and animal for food resources 2. Development of strategies, using feed additives, to reduce possible negative effects of intensive animal agriculture on the environment, as for example the use of phytase to reduce phosphorus excretion to water bodies or in the manure 3. Improving efficiency of utilisation of finite resources by studying of alternatives that meet animal need without jeopardising animal growth and productivity 4. Understanding of alternatives to antimicrobial growth promoters to ensure optimum growth of birds and reduce subclinical growth performance issues
What species and approximate numbers of animals do you expect to use over what period of time?	Species to use are broilers, ducks and turkey Nutrition efficacy studies: 10,000 Feed evaluation – gavage: 300 Feed evaluation – raised floor: 5,000 Foot and hock studies: 2,000 Standardised digestibility studies – 4,000 Nutrition and gut health studies – 4,000
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some of the birds in the project will receive diets that are not meeting nutrient requirement (vast majority of the birds will receive diets with adequate nutrients) and such birds will be expected to gain weight more slowly. In establishing the standardised digestibility, some of the birds in the experiment will be provided with diets with very little protein or mineral for a very short period, not exceeding five days. The potential negative effect is reduced growth, but in order to reduce this effect, the treatment will only be applied to birds that have received diets that are adequate in nutrients for at

	least 7 days. Oral gavage of birds with campylobacter or coccidia may produce reduced growth rate. However, this is to mimic what may happen in a typical poultry farm, and the negative effect will be minimised by ensuring that birds that are orally gavaged with the organisms are kept only up to 10 days after induction. The maximum severity limit in the project is moderate but most of the birds to be used in the project will have mild severity level. At the end of the experiments, some of the birds will be euthanised using humane methods. Some of the procedures do not require euthanasia of birds as part of the experiment and such birds will be signed off the Act.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studies that require effect of treatments on growth can not be done in non-animal substitute. Digestibility studies are usually preceded by in vitro proof of concepts but ultimately because the feed additives will be incorporated to feed for actual animals, it is a requirement that such products are fed to animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of replications and animal per replications are determined based on statistical analyses and previous experience. Each experiment is individually set up to maximise ability to test for treatment effect combined with every effort to use the minimum number of animals. Experimental protocols are reviewed before each experiment to ensure that animals are not used unnecessarily. In addition, there are monitoring exercises after experiment to see what lessons are learnt. The 3Rs is part of the monitoring exercises. Part of the review of the experiment include the input of expert statistician (BiOSS)
3. Refinement Explain the choice of species and why the animal model(s)	The objective of this project is to provide information that is relevant to the poultry industry, and hence poultry species are the choice of animals for the project. There are instances where

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.	nutritional effects studied are applicable across species (i.e. what is studied in broilers may be applicable to turkey) and in such cases, studied are not repeated for all the poultry species. Most of the protocols in this project are mild in severity level. Birds used in this project will be monitored on a daily basis to ensure that birds wellbeing is not compromised. There is also on-site specialist (avian) veterinary support who helps ensure birds wellbeing is maintained.

<table>
<tr><td>PROJECT 14</td><td>Development of Poultry Coccidiosis Vaccines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Poultry Coccidia Eimeria Vaccine</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[21]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[22]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To support the research, development and production of safe efficacious Coccidiosis vaccines for poultry.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>1. Improvement of the efficacy of existing Coccidiosis vaccines.
2. Reduction of the number of birds required to produce live chicken coccidiosis vaccines, compared with current methodology.
3. Feasibility of a cost effective Coccidiosis vaccine for use in broilers thus providing a safer alternative to the chemical control currently used in the poultry industry</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over the 5 year lifetime of the licence:

3,500 embryonated chicken eggs</td></tr>
</table>

	10,500 chickens 500 turkeys
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 clinical signs associated with Coccidiosis in poultry are mild and non-specific and include* depression, prostration, and huddling. More severe disease including soiled vents and watery or bloody droppings is however associated with infections involving *E. tenella or E.necatrix. I*t is recognised that the outcome of infecting such birds with these species can however be unpredictable and may lead to rapid deterioration and death. Whilst challenge doses will have been selected to minimise the likelihood of this occurrence and the frequency of observation will be increased to monitor for the humane end point prevention of a severe outcome cannot be guaranteed. 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	The test requirements for regulatory purposes are defined by the European Pharmacopoeia monograph 04/2013:2326 and there are currently no non animal models available to demonstrate the safety or efficacy of chicken. The use of laboratory culture systems for the growth of *Eimeria* parasites is limited because current techniques do not allow for reproducible completion of the complex lifecycle or the production of consistent high yields of oocysts.
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 European Pharmacopoeia monographs and other regulatory guidelines. For other studies the protocols will be designed to combine the collection of data on as many different parameters as possible within a single study, subject to the severity of the procedures carried out, in order to minimise the number of animals required. A qualified statistician will provide advice to help to determine the minimum group sizes required to generate

	statistically meaningful data.
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.	*Eimeria* parasites are highly species specific and it is therefore only possible to achieve the objectives of this project using chickens and turkeys. All studies are planned in advance and the study plan reviewed by the R&D project team, R&D management, NVS, NACWO and quality assurance colleagues. Where possible, the animal model and procedures to be used reflect extensive in-house expertise, peer-reviewed published protocols and /or defined regulatory requirements. Further refinement is encouraged through pre-study briefings with all active stakeholders and a post-study review process. Clearly defined humane end points will be detailed in the protocols and where more severe clinical signs are anticipated the frequency of observation will be increased during any critical period. Birds will be euthanised immediately the humane end point has been reached.

<table>
<tr><td>PROJECT 15</td><td>Testing of Chemicals on fish for use offshore</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ecotox testing – Juvenile fish</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[23]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[24]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Good quality data for regulatory purposes to allow only chemicals to be used off shore to protect environment.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Environment protected from unsafe chemicals</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The recommended species are turbot juveniles (Scophthalmus maximus - minimum of 7 per group) and Sheepshead minnow juveniles (Cyprinodon variegatus - minimum of 10 per group).

The recommended species for freshwater tests are</td></tr>
</table>

	Brachydanio rerio (Zebra Fish) and Oncorhynchus mykiss (Rainbow trout), The approximate number would be 16,650, over the five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Fish are exposed to test compounds by adding those compounds to the water that the fish are held in. The vast majority of chemicals do not cause any effects, because they have already been screened by non-animal tests to ensure that only compounds expected to be non-toxic are tested in fish. Very occasionally fish show signs such as loss of equilibrium, erratic swimming behaviour and respiratory function where this occurs they are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are bound by the regulatory authorities who decide upon the guidelines that are appropriate for fish testing on chemical products to be discharged into the environment. The regulatory authorities state that it is mandatory to carry out a fish test on all oil field chemicals or their components.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Regular correspondence with operators to determine whether fish testing is required. In March 2006, limit tests were introduced to reduce the number of fish used for toxicity tests. Guidelines were amended to carry out a limit test(single concentration) if there was no existing fish toxicity test data, the concentration was to be conducted using invertebrate and algal tests, whichever is the most sensitive, this resulted significantly in a reduction of the numbers of fish being tested.
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	The choice of the fish species is determined by the regulatory authorities, where survival is expected after 96 hours in a single limit test concentration or mortality is the endpoint. If the fish show signs of significant loss of equilibrium, erratic swimming behaviour,

measures you will take to minimise welfare costs (harms) to the animals.	respiratory function, pigmentation and mortality, then they are removed from the test tank. The fish are humanely killed by an overdose of anaesthetic by immersion as stated in the Schedule 1 Method.

<table>
<tr><td>PROJECT 16</td><td>Inhalation Toxicology - Chemicals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inhalation, Aerosols,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[25]</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[26]</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The evaluation of chemicals for effects, following administration by the inhalation route, using methods which comply with the published testing guidelines (acute and repeat dose).

Such studies are only required when it can be demonstrated that the substances in question has the ability to present an inhalation hazard to humans and suitable data do not already exist.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The results of these studies allow regulatory bodies to control the manufacture, transport and supply of new products and to make the necessary risk assessment for human and environmental exposure.

The results of these risk assessments enable the appropriate risk management strategy to be</td></tr>
</table>

	enacted and this can include the appropriate classification, labelling, hazard communication, transport limitations or banning of substances. For clarification; the justification for animal based regulatory toxicology and safety testing is the need for regulatory authorities and/or manufacturers to have sufficient information to assess the risks to which humans, animals, plants or the environment are exposed when the test substances are produced, transported or used. Thus, provided a legal requirement can be demonstrated, the principal benefit is the facilitation of sound regulatory decisions.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat (adult)/Mouse (adult) – Approximately 4000 over a five year time 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?	<u>General Effects of Substances - Acute</u> Exposure to test substances can cause a range of effects ranging from mild, transitory respiratory rate effects to mortality. <u>General Effects of Substances – Repeat Dose</u> Dose-levels are set so as to produce clear indicators of toxicity at the highest exposure concentration without seriously affecting the well-being of the animals, the maximum severity for these protocols will therefore be Moderate. Animals that are considered unlikely to survive or are obviously in pain or showing signs of severe distress will be humanely killed in a timely manner. All humane kills are recorded. Criteria for making the decision to humanely kill animals are characterised by signs of toxicity that may be predicted to culminate in greater than moderate toxicity. These may include respiratory pattern changes, significant loss of weight (bodyweight loss of more than 15% over a period of 7 days), evidence of any signs of hypothermia, pallor, dehydration, reduced voluntary response to external stimuli and abnormal changes in posture and locomotion will results in a greater frequency of monitoring, if these are then considered to be causing significant animal welfare harms these animals will be humanely killed. It is predicted that these types of clinical signs would be noted in a relatively small percentage of animals on repeat dose studies as the objective of the studies is not to cause a severe affect on the well being of the

	animals. In cases of where animals appear to be suffering more than moderate pain or discomfort, or where this is prolonged, or appear distressed, animals will be humanely killed regardless of the impact on the study. <u>Exposure</u> Restraint during exposure (nose-only exposure) may cause stress and mild discomfort to some animals. Care will therefore be taken to ensure that the minimum possible restraint is used whilst ensuring the animals cannot avoid the test atmosphere. All animals will be humanely killed at the end each separate study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are currently no in vitro methods suitable for the replacement of these protocols, nor are there, as yet, any accepted protocols to enable further reduction in the severity of these studies or in the number of animals used. If the data are not required for formal submission as part of a regulatory package, it is sometimes possible to perform a screening study using reduced animal numbers in order to show the test item is/is not harmful/toxic. The studies investigate a wide range of end-points (including pathological examination of direct tissue or cellular effects in a wide range of target tissues each composed of multiple cell types) and examine more subtle toxic effects (including behavioural modifications and neurotoxic changes). The complex interactions involved in systemic toxicity are such that these effects can, currently, only be effectively studied in live animals.
2. Reduction Explain how you will assure the use of minimum numbers	When the test item is expected to be relatively non-toxic the OECD 403 and 436 test guidelines allow the use of a reduced number of animals at the limit test target concentrations of (3 males and 3

of animals	females or six animals of the most susceptible sex). For all dose groups for the OECD 436 test guideline the total numbers of animals that are used for a full study (in order to determine classification of the test item) is markedly reduced in comparison to the other test guidelines and as such this is now the preferred method to be used within this establishment unless there is a specific justification for a definitive LC50 can be provided.
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.	OECD 403, OECD 436 and other test guidelines specify the use of young adult rats, other rodent species may be used (including the mouse) but the rat is the species of choice. For the OECD 403 - Current requirements demand an LC_{50} determination and so it is necessary to expose some animals to lethal concentrations and mortalities and marked signs of toxicity are, therefore, expected in a number of studies performed under this protocol. Where it is clear that animals are showing signs of non-recoverable toxicity, they are humanely killed and the aim is to intervene and not to have death as an endpoint. As studies progress, better estimates on the possibility of recovery from the effects of exposure, based on previous exposure groups, can be made and therefore it becomes easier to determine humane endpoints. For the OECD 436 – this test guideline requires exposure of some animals to lethal concentrations and mortalities and marked signs of toxicity are, therefore, expected. From experience these kinds of affects may be noted in approximately 20 % of studies performed under this protocol. As studies progress, better estimates on the possibility of recovery from the effects of exposure, based on previous exposure groups, can be made and therefore it becomes easier to determine humane endpoints. Refinement is also achieved by systems of care and accommodation that enhance the animals' welfare. Environment enrichment by the use of fun tunnels, chew blocks etc is provided. Group

	housing of social animals is encouraged unless precluded on scientific grounds.

<table>
<tr><td>PROJECT 17</td><td>Toxicology: Medical Products</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Regulatory Toxicology Safety Assessment</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[27]</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[28]</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The assessment of new or existing medical products to determine the safety to man and/or animals. The primary objective is to identify the potential hazards to human health associated with exposure to such medical products and provide the information required to allow for the determination of risk to human/animal health associated with exposure to such medical products.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This Laboratory has a long established record of Toxicology testing of new chemical entities. Our experience in identification and reporting of hazards give confidence to subsequent risk assessment thereby allowing appropriate decisions to be made regarding the safe us of new Chemical products</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Rats/Mice – 10 000 over a five year period Rabbits – 1000 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?	For the majority of studies; the intention is to see all animals to complete the expected treatment/observation period. The use of dose range finding studies with small groups of animals and data from studies with comparable materials will ensure appropriate dose level selection for main studies. The overall majority of animals on this project are expected to show no more than moderate signs of severity in response to treatment with common clinical observations being slight pilo-erection, transient respiratory changes plus slight reductions in food consumption, increased/decreased water intake and a slight reduction in weight gain over the study. The objective is to fulfil regulatory requirements by use of the maximum tolerated dose. The exception is the use of acute toxicity tests where less information is known and less confidence can be applied to dose level selection. For this reason these procedures are considered potentially severe because there is more risk of an adverse reaction to treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As part of national and international legislation it is a requirement to conduct safety evaluation studies using prescribed methodology which has worldwide acceptance. Non –Animal methodology can and will be used if these can achieve the necessary information and the methods have received approval for use from the appointed national/international bodies responsible for product approval
2. Reduction Explain how you will assure the use of minimum numbers of animals	The methods used follow the appropriate testing Guidelines as advocated by National/International bodies responsible for New Product Approval. The use of more than the minimum prescribed will be subject to approval by the appointed Inspectorate

	and will be determined by the scientific need for such additions
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 choices of species are those prescribed in the testing guidelines for ICH and OECD. From any years of use, the response to exposure to test materials are well understood and allows us to make informed decisions regarding care and welfare of test models. Well-designed dose range-finding studies allow correct dose level selection and main study design

PROJECT 18	**Genetic Toxicology – Chemicals**	
Key Words (max. 5 words)	Mutagen, Genetic Toxicology	
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
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals[29]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective is to investigate if positive *in vitro* test responses are confirmed the relevant *in vivo* test method. This will allow sponsors identify if test substance is to be labelled as a mutagen. In some instance the in vivo studies are required when there is a risk of possible high human exposure, substance use in food contact materials for instance.	
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 general consensus is that most mutagens have the potential to be carcinogens. Therefore the potential benefits of the project are the identification of *in vivo* mutagens which may induce cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice are recommended species in all the OECD test methods we use. During life span of the project licence we expect to use 9000 mice and 3000 rats.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Expected adverse effects are those associated with the method of administration and the required level of handling this will require, and any toxic response to the test substance exhibited by the animal. Care will be taken to ensure that the latter is kept within the severity limits set for the main test protocols. The aim is not to exceed a moderate severity limit.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present there are no validated non-animal alternatives available that can satisfy the regulatory authorities for this area of hazard identification.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initially by ensuring there is a valid reason to perform the study. Reviewing all available toxicity data to ensure the initial dose levels used in the rang-finding test are not excessive. Performing all main tests using a single sex if there is no marked difference in toxicity, and getting a valid scientific justification form the Sponsor if they want both sexes to be used. If the sponsor is looking at performing two in vivo studies look at performing them in a combined experiment. If possible bolting on a genetic toxicology end-point onto a repeat dose toxicity study.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Both rats and mice are recommended species in the relevant OECD test guidelines. There are many years of research publication demonstrating the suitability of these species in these test systems. By careful analysis of all toxicity data available on all test substances the initial dose levels used in the range-finding tests will be set to cause as little harm as possible. Careful monitoring post dosing will also ensure any excessive welfare issues adverse effects are terminated quickly.

<table>
<tr><td>PROJECT 19</td><td>Collection of body fluids and/or tissues for in vitro use</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Body Fluids/Tissues In-Vitro Use</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[30]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[31]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall objective is to provide scientists working for or in collaboration with the Establishment, with animal body fluids and/or tissues of appropriate quality to support the research and development of new medicines.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The potential benefits of work performed under this licence include: development and validation of in vitro assays to replace testing in live animals, calibration/validation of technical instrumentation and quality assurance checks, reduction in the total number of animals required by centralising the supply of body fluids and tissues (i.e. by use of a tissue bank, where possible).</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of this 5 year project we expect to use: 100 guinea pigs; 250 hamsters; 1800 mice and 2250 rats (normal and genetically altered); 100 rabbits; 250 dogs and 50 pigs.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of samples collected under this licence will be blood and organ/tissue samples. Other samples, such as urine or oral swabs, will be collected more rarely. The majority of techniques will be performed in anaesthetised animals. Typically, samples will be taken from rodents (e.g. blood, tissue samples) as part of a non-recovery procedure with the animal unconscious and euthanased at the end of the procedure. Samples from dogs, pigs and rabbits (e.g. blood or urine) will often be taken whilst the animal is conscious. Procedures that are performed on conscious animals are unlikely to cause pain or distress of more than minimal and/or transient nature. Sometimes samples will be taken from animals that have been used previously. The general state of health and well-being of these animals will have been assessed by the Named Veterinary Surgeon as fully restored following the previous procedures. Some of the rodents (approx 10%) will be genetically altered (GA). Most of these are expected to show minimal effects of the alteration, (e.g. hair loss), and experience mild severity. Rarely GA animals that may exhibit more significant phenotypic effects to moderate severity, (e.g. premature aging), will be required The severity, experienced by the majority of the animals, is expected to be mild. Any animal showing adverse effects will be monitored closely and veterinary advice will be taken or, if appropriate, the animals euthanased.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible studies will be performed on human tissues/ fluids or on cell lines. Studies requiring animal body fluids or tissues are only performed when all suitable in-silico and in-vitro alternatives have been exhausted. Samples will only be taken from animals after a set of questions have been answered by the requestor and assessed as satisfactory by the Project Holder or his delegate. Questions will include checks that the work requested fits the purpose of this licence, alternatives to animals have been considered and judged to be unsuitable, and animal use is justified as necessary for the research. The provision of animal body fluids and/or body tissues will aid the development and validation of in vitro assays that replace live animal tests. Additionally, the identification of suitable species in which to carry out target validation, the development of biomarker assays and requirements for regulatory testing in animals requires validated assays.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Ex-vivo studies will be designed to ensure the minimum number of tissues / volume of body fluids are required and therefore the minimum number of animals will be used consistent with achieving the objectives of the study. Frequently larger samples than those required to meet the immediate objective of the work, will be taken, and retained for future use as part of a tissue bank. This will reduce the number of in- vivo procedures performed on animals; however, larger volumes will not be taken if the harm to the animal is increased. Wherever possible multiple samples will be taken from a single animal during a non-recovery procedure to provide tissues for multiple projects. The Establishment has proven systems e.g. electronic tissue request/distribution lists that

	facilitate efficient use of samples. Where appropriate, we will retain remaining tissues/body fluids for future use in other in vitro 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 species used will be determined by the scientific requirements of the new medicines discovery and preclinical programmes. Samples from rodents will be the first choice to fulfil the needs of these projects. In approximately 10% of studies non-rodent samples may be essential to ensure successful development of an assay or progression of a Project. Immunocompromised or Genetically Altered (GA) animals will be used to support programmes of work when scientifically justified. All experimental procedures have been subjected to ethical review and are conducted by highly trained, competent scientists. A Named Veterinary Surgeon and Named Animal Care and Welfare Officer is available for advice on study design including appropriate use of anaesthetics and analgesics and animal welfare matters. Environmental enrichment and socialisation are used widely. Animals are group housed wherever possible unless there is a welfare or scientific justification for single housing, e.g. to prevent animals fighting.

<table>
<tr><td>PROJECT 20</td><td>ADME and incurred tissue studies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>ADME, veterinary, livestock, incurred residues</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[32]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[33]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of the project is to carry out studies:
1. On the Adsorption, Distribution, Metabolism and Excretion (ADME) of veterinary medicines in food producing animals
2. To supply animal tissues containing tissue-bound (incurred) residues of veterinary medicines for use in scientific projects carrying out research, method development, method validation, quality control (QC) and performance assessment.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>ADME studies are part of the regulatory requirements which have to be met before a veterinary medicine can be approved to use in a particular species. ADME studies are part of the information required to ensure the safety of consumers exposed to food products derived from animals to which the veterinary drug has been given.

In addition to issuing approvals for the use of veterinary medicines, regulatory authorities also conduct monitoring surveys of veterinary medicine in food (e.g. in tissues from food-producing animal). This monitoring is designed to ensure that only approved veterinary medicines are being used and that approved medicines are being used in the correct manner. To conduct this monitoring, analytical methods to determine residues in tissues are required. The validity of analytical measurements is critical, since important decisions</td></tr>
</table>

	are made based on them. EU legislation (Commission Decision 2002/657/EC) requires that new analytical methods are developed and validated using tissues containing incurred residues. The use of incurred tissue also allows laboratories to assess and monitor their performance, both short and long term and to identify problem areas. Better, more valid, more robust and better understood chemical data, produced with the use of incurred tissue, will allow regulatory bodies to make better judgements on the risks associated with residues and help to safeguard human health and protect the food chain.
What species and approximate numbers of animals do you expect to use over what period of time?	Total numbers of animals used over the duration of the project are listed below with expected numbers per year indicated in brackets Pig, juvenile & adult: 500 (100 per year) Chicken, juvenile & adult: 800 (160) Turkey, juvenile & adult: 800 (160) Cattle, juvenile & adult: 160 (32) Sheep, juvenile & adult: 210 (42) Goats, juvenile & adult: 110 (22) Rabbits, juvenile & adult: 100(32)
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 animals to be used are all food producing animals, the tissues of which are eaten by consumers. The animals will, after a period of acclimatisation, be given one or more doses of veterinary medicine, either in their feed, or in solution (in some cases the solution will con in a capsule, or by injection if this is the normal route by which the medicine is administered. The likelihood of any adverse effects is generally very low since the veterinary medicine will be used at normal therapeutic levels. Where possible, administration of substances is carried out by addition to feed or water to minimise any distress arising from handling or administration. Extensive veterinary involvement and advice will be used to further minimise side effects The administration of substances as liquids (either as encapsulated doses or oral dose) will only be carried out by appropriately trained staff. Consultation with a veterinary surgeon will ensure that appropriate

	sizes of capsules / dose volumes/rates will be used. It is not anticipated that such methods will result in any adverse effect beyond transitory distress at the time of administration. Where the veterinary medicine needs to be injected, this will only be carried out by appropriately trained staff. Consultation with a veterinary surgeon will ensure that appropriate sizes of needle and injection volume/rate are used. It is anticipated that such methods will result in transitory distress at the time of administration and short-term pain/discomfort at the injection site. It is NOT anticipated that the injection technique, in and of itself should cause any additional pain, distress or harm. At the end of each trial, the animals will be killed humanely and organs and tissues collected for analysis.
Application of the 3Rs	
1. Replacement *State why you need to use animals and why you cannot use non-animal alternatives*	The biochemistry of vertebrates is complex, and substances pass through different organs and tissues before being deposited or excreted. Within each one, many chemical and biochemical processes may occur. These do not occur in isolation, and many substances undergo a variety of them. Hence techniques like tissue culture are unsuitable, since they address only a single tissue, often not one of regulatory interest. Further, tissue culture and similar techniques cannot produce the quantities of tissues required for use in chemical processes. Analytical methods require large samples (2-10g), so significant quantities of tissues are needed. Only animal studies can combine the requirements of the complexity of the system, the applicability of the product and the volume of material required.
2. Reduction *Explain how you will assure the use of minimum numbers of animals*	ADME studies will only be conducted when appropriate data of suitable quality is not already available for a particular veterinary drug compound in the species of interest. A detailed study plan will be produced for each study which will contain the number of animals to be used. The study plan will be reviewed against the relevant guidelines to ensure that the study will not have to be repeated

	because minimum requirements for animal numbers were not met and to ensure that excessive numbers of animals are not being used. For procedures designed to produce incurred residues for validation of analytical methods, the minimum number of animals that will be required to generate a given mass of material will be used. This can be estimated accurately from the expected bodyweight and the proportion of a given organ / tissue within each animal or from the expected rate of production of materials such as milk or eggs.
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.*	Typically, animals (pigs, chickens, turkeys, sheep, cattle, goats, or rabbits) will receive veterinary medicines or related compounds at normal dose levels and durations, via normal routes of administration. The animal species listed are all used in the human food chain. Hence, these are the most suitable to carry out work on incurred residues in animal tissues that enter the human food chain. The species to be used in each ADME study will be the species for which approval to use the veterinary drug is being sought. For studies to produce incurred residues for validation of analytical methods, the species to be used in each particular study will depend on the species for which analytical methods need to be validated. This, in turn, will depend on the species in which residues of a particular compound have been detected in monitoring programmes of veterinary drug residues in tissues e.g. if monitoring programmes show that residues of a particular veterinary drug as being detected in chicken tissues, and there is a need to generate chicken tissues containing incurred residues of that compound, then chickens will be used. As far as possible, all protocols are designed to reduce the pain, distress and harm suffered by the animals under test. This includes dosing and treatment via feed or water wherever possible, minimising invasive treatments and regular, in-depth veterinary involvement designed to mitigate any and all adverse effects to the greatest possible degree. Animals are, wherever possible housed with conspecifics in purpose built facilities, and are always within sight and sound of conspecifics. Inspection / checking regimes are thorough to

	ensure that in the event of any animal suffering adverse effects; these will be noted (and hence treated) as rapidly as possible, and the Home Office will be notified.

PROJECT 20	**Effects of noval and other compounds on GI tract**
Key Words (max. 5 words)	Gastro-intestinal system, Safety, Pharmacology, Efficacy.

- Summarise your project (1-2 sentences)

The aim of this programme of work is to determine the safety and efficacy of substances (predominantly novel pharmaceuticals) that may interact with the gastro-intestinal system.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Non-compliance, whereby patients either refuse treatment or stop treatment prior to completing the planned course is a common problem and can represent a serious risk to the individual. A major cause of patient non-compliance is the patient's intolerance of a drug due to side effects caused by gastro-intestinal disturbances. It is therefore important, where possible to produce drugs that are well tolerated and as free as possible from such side effects. The majority of work conducted under this Licence will be concerned with side effect profiling with the ultimate aim of minimising side effects such as constipation, diarrhoea, emesis, excess stomach acid production (a major cause of ulcers) and gastric damage. In addition, before humans are exposed to new substances, their safety must be evaluated; this is a mandatory legal requirement as will as an expectation by the public.

When patients are treated with drugs that are known to produce gastro-intestinal side effects that cannot be avoided, such as emetic activity caused by substances used in chemotherapy, it is important for the quality of the patient's life to reduce these effects as far as possible. This Licence therefore also allows for efficacy testing that will, for example, assess potential useful drugs affecting the gastro-intestinal system. This may involve trying to inhibit or reverse the activity of a known pharmacological agent with a predictable gastro-intestinal side effect.

- Outline the general project plan.

This project licence will enable the assessment of gastro-intestinal function. As previously stated, the work will predominantly focus on side effect profiling although the assessment of efficacy of putative drugs with a desired effect on the system may also be evaluated.

Both aims can be achieved using similar assessments. To take emesis as an

example, the scientific endpoint will be to visually assess the emetic response in the animal. In terms of safety, the substance under investigation will be administered to the animal which will then be assessed for emetic activity. For an efficacy evaluation, in which the test substance is being assessed for the ability to inhibit or reverse emesis, the substance will be administered in conjunction with a compound known to cause emesis. The observation of the animal will again be for emesis but this time, animals are likely to be sick if they have received the emetic compound alone but if they have also received the test substance at an efficacious dose, the emesis should be inhibited or abolished altogether.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

The majority of studies conducted under this licence will be short term, usually using assessments following a single dose of the test substance. Therefore, It is envisaged that studies should not cause more than a period of abdominal discomfort in most cases. For efficacy studies when animals are also given an additional compound to elicit an effect against which the test substance is assessed, an effect on some animals in the study is expected. Again these are, in most cases expected to cause abdominal discomfort in most cases or limited periods of emetic activity from which the animals recover within a short period of time eg 4 hours.

Specialist veterinary staff are always available to advise and assist in the welfare of the animal. Humane endpoints are applied, under veterinary guidance.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

Novel drugs may be developed with reduced or limited gastro-intestinal side effects or a side effect profile that may be better tolerated than currently marketed products. Alternatively, novel drugs that produce beneficial effects via actions on the gastro-intestinal system may also be assessed as part of this licence.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

Mice and rats will predominantly be used in these studies. These species are used because they respond to compounds active in the gastro-intestinal tract in a similar manner to the humans and the data produced will help predict how well the potential medicines will work in humans. In a limited number of studies, ferrets may be used as the rodent does not possess a vomiting reflex and is therefore inappropriate for the assessment of emesis. In rare cases, non-invasive technology in dogs may be used to replace work in animal models that would, without such technology, result in

the death of the animal.
• Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project. The number of animals used will be kept to a minimum and statistical analysis will be used to determine the number of animals required per group to promote a meaningful outcome. Experimental designs are constantly reviewed and alternative cell assays considered as technology improves, however due to the complex nature of the gastro-intestinal system there are no current alternatives to use animals.
• Explain why the protocols and the way they are carried out should involve the least suffering. Wherever possible, experimental end point data is collected under anaesthesia or post mortem to reduce the suffering of the animals used in the protocols. In some circumstances safety markers will also be collected from the animal models maximizing the data from individual studies. This will be considered when the additional impact on the individual is small whilst negating the necessity to use additional animals.

PROJECT 22	**Aquatic Ecotoxicology**		
Duration of project	5 years		
Key Words (max. 5 words)	Regulatory, toxicological, data, environment		
Expected duration of the project (yrs)	5 years		
Purpose of the project (as in Article 5)	Basic research		No
	Translational and applied research		No
	Regulatory use and routine production	Yes	
	Protection of the natural environment in the interests of the health and welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals, not used in other procedures		No
Describe the Objectives of the Project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To provide government regulatory agencies with the ecotoxicological data, they have stipulated necessary, for a balanced assessment of the potential impact of new and existing chemicals on the aquatic environment and its ecology (e.g. fish).		
What are the potential benefits likely to derive from this Project (how science could be advanced or humans or animals could benefit from the project)?	The introduction of new chemicals, such as drugs, biocides or agrochemicals, is generally considered to be beneficial because of the associated improvements in the quality of life. New medicinal products can alleviate diseases and handicaps, new plant protection products can optimise crop yields and improved industrial chemicals can facilitate production of manufactured materials with greater utility and economic advantage.		
	A range of freshwater (rainbow trout, fathead		

What species and approximate numbers of animals are expected to be used?	minnow, medaka, bluegill sunfish, zebra fish, common carp, brown trout and stickleback) and marine (turbot, sheepshead minnow) fish species; maximum number per test type over the duration of the project ranging from approximately 400 to 81,000.
In the context of what is being done to the animals, what are the expected adverse effects on the animals, the likely/expected level of severity and the fate of the animals?	Acute testing protocols have severe severity, and are conducted in accordance with OECD test guidelines, which specifically request that, where possible, the concentration that kills 50% of the fish (LC_{50}) is determined. Marked toxicity requiring euthanasia, and/or death at higher concentrations may be expected in these tests. With the exception of partial/full-life cycle tests, chronic testing protocols have mild severity and are designed to determine non-lethal effects of chemicals on fish. For partial/full-life cycle tests, the parental generation are exposed to sub-lethal concentrations, but this may result in effects in subsequent generations (e.g. larval effects) that may be categorised as severe. For all protocols, where an animal displays significant effects and distress following exposure to a chemical, it will be removed from a test and humanely killed quickly and efficiently. All surviving animals at the end of toxicity tests are humanely euthanised.
Application of the 3Rs	
1. Replacement State why animals need to be used and why non-animal alternatives cannot be used	Ecotoxicology studies, as an integral part of the safety evaluation process, help to ensure new and existing chemicals do not present a health hazard to man and his environment. These studies are a key element in the environmental risk assessment of these chemicals and at present there are no official in-vitro alternatives recognised by international regulatory authorities (e.g OECD, US EPA etc) that provide suitable results.
2. Reduction Explain how the use of	Prior to initiating testing with fish, a literature search and application of structure activity relationships (SAR) will be conducted to determine whether

minimum numbers can be assured	suitable GLP compliant information is already available for the specific test substance or formulation. If the occasion occurs where suitable data is available for client use, testing may not be required and therefore clearly reduces the number of animals used. Where information on a chemical is available and suggests that there would be no or limited toxic effect then a limit test may be performed. In a limit test, a reduced number of fish would be used. Where data are available for another fish species, it may be possible to use this information to determine a concentration range to be used for a definitive test and therefore, there would be no-requirement for a range-finding test to be conducted. Where information is available on a particular chemical but generated through testing on another test species, such as an aquatic invertebrate (*Daphnia magna*), it may be possible to use the information generated in this alternative test and avoid testing on fish altogether. This approach is becoming more acceptable amongst regulators but requires discussion with the appropriate regulators to assess acceptability before use.
3. Refinement Explain the choice of species and why the animal model(s) used are the most refined, having regard for the scientific objectives Explain the general measures to be taken to minimise welfare costs (harms) to the animals.	The regulatory guidelines recommend which species should be tested. The species have been used extensively. Their response to chemicals is very well known. Typically, the recommended species tend to be those that have a greater sensitivity to chemicals, which effectively safeguards less sensitive species in the environment. Where an animal displays significant effects and distress following exposure to a chemical, it will be removed from a test and humanely killed quickly and efficiently. The decision to remove and humanely kill an animal from a test is made by appropriately trained members of staff with the full support of the Study

	Director and the Project licence holder as required. Professional advice may be sought from the NACWO (Named Animal Care and Welfare Officer), the NVS (Named Veterinary Surgeon) and the Home Office Inspector as required. All surviving animals at the end of toxicity tests are humanely euthanised.

<table>
<tr><td>PROJECT 23</td><td>Assessing the Risk of Environmental Contaminants to Fish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish Environment Risk</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[34]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[35]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The generation of high quality data regulatory risk assessments and for the assessment of chemical mixtures that can be found in the environment.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The risk assessments are made to help with decisions for the proportionate regulation of chemicals that are discharged to natural waters while protecting wild fish and the wider aquatic ecosystem.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The species selected will depend on the purpose of the test, i.e. whether it is specified by a regulatory method or whether it is representative of fish in UK water bodies. The optional species are Trout, Stickleback, Fathead Minnows, Zebra Fish and Rice Fish. Up to 10,000 fish may be used over a five year period.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>In the majority of cases, the effects are likely to be moderate, i.e. the fish will behave normally, but reproduction or growth may be affected. In some cases, the effects may be severe, i.e. the fish will show strong signs of sickness. These fish will be killed as soon as this is seen. All the fish used will be humanely killed at the end.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
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1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tests using fish are currently required by regulators and are considered the only way to be sufficiently certain that wild fish will be protected by the results of the risk assessment. However, research is on-going to look for alternatives ways of being equally sure of protecting wild fish without testing fish in laboratories (e.g. by using living cells).
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of fish that is allowed by the regulatory guidelines to give credibility to the statistics used to calculate the results of the risk assessment will always be used. It will also be established that any testing has a clear need for the protection of wild fish in the aquatic environment.
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 species used are those specified in the regulatory guidelines. Sometimes there is a choice, and in these cases, the species that is the most representative of the wild fish in the environment to be protected are chosen. All of these species prefer to live in groups, and so no fish will be isolated. Fish are also stressed by disturbance and handling and so these will be kept to a minimum. Any sick fish will be humanely killed, as will any larvae that hatch out with strong deformities.