# Animals (Scientific Procedures) Act 1986

## Non-technical summaries for project licences granted during 2015

## Volume 1

Projects with a primary purpose of:

- Protection of the natural environment in the interests of the health or welfare of human beings or animals
- Preservation of species

**Project Titles and keywords**

1. **Improving livestock production efficiency, quality and safety**
    - Livestock Production, Product Quality, Environmental Protection, Product safety

2. **Determining red fox movement patterns around breeding birds of conservation concern**
    - Fox, tagging, collar, capture, conservation

3. **Acoustic Fish Tracking in Tidal Waters**
    - Acoustic tracking, Salmon, Trout, Eel

4. **Ecology of small carnivores and their prey**
    - Wildlife conservation, predator-prey, ecology

5. **Detection of *C. botulinum* spores/toxin**
    - Clostridium, botulinum, toxin, spores, bioassay

6. **Chemicals and fish research**
    - Toxicity testing, environmental pollutants

7. **Assessment of medicines for the treatment of external parasites of farmed fish**
    - Salmon, Sea bass, parasites, medicines

8. **Assessing the risks to fish of environmental stressors**
    - Fish, risk assessment, chemicals, environmental stressors

9. **Lungworm Vaccine Primary production**
    - Dictyocaulus viviparous

10. **Studies of virulence in human and animal fungal infections**
    - Fungi, virulence, infection

11. **Exposure of animals to animal viruses for diagnosis and characterisation**
    - Animal, Viruses, Diagnosis, Characterisation

12. **Effects on metabolism and endocrine pharmacology**
    - Metabolism, Endocrine, Safety, Efficacy, Pharmacology

13. **Environmental Toxicity, Metabolism and Fate of Materials**
    - Ecotoxicology, Metabolism, Fate

14. **Monoclonal and polyclonal antibody production**
    - Monoclonal, polyclonal, antibody

15. **The fitness and evolutionary consequences of environmental variation**
    - Selection, environmental change, diversity, speciation

16. **Water pollution and salmon magnetoreceptivity**
    - Atlantic salmon, olfaction, magnetoreceptivity, migration, water pollution

17. **Examining dietary specialisations in buzzards**
    - Predation, dietary specialisation, buzzard, isotope

18. **Ecology of dormice**
    - Hazel Dormice, habitat management, radiotracking, forestry

19. **Avian malaria in migratory and resident birds**
    - Migratory bird, resident bird, blood parasite, avian malaria

| **Project 1** | **Improving livestock production efficiency, quality and safety** | |
|---|---|---|
| Key Words (max. 5 words) | Livestock Production, Product Quality, Environmental Protection, Product safety | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | X | Basic research |
| | X | Translational and applied research |
| | | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | This project seeks to examine ways of optimising efficiency of ruminant meat production from predominately forage-based systems. It will examine methods of enhancing product quality, particularly in terms of healthiness, shelf life, hygienic quality, flavour, at the same time as reducing the impact of these systems on the environment.<br><br>The research will also contribute towards the production of healthier food in terms of a leaner product and meat which will contain lower quantities of saturated fatty acids and increased content of specific beneficial compounds (i.e. n-3 PUFA linolenic acid, minerals e.g. Se) in meat. The role of meat as a vehicle to deliver beneficial fatty acids and micro-nutrients through to food products is very important. Food borne pathogens such as E. coli O157 can be fatal to the weakest members of our society; this proposal will provide fundamental knowledge which will help us identify practical methods to prevent | |

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| | infections in animals and humans.<br><br>Methane produced during anaerobic fermentation in the rumen represents an energy loss to the host animal as well as contributing to emissions of greenhouse gases into the environment. On a global scale agriculture and in particular enteric fermentation in ruminants produces between 21 and 25% of the total anthropogenic emissions of methane. Over the last 30 years ionophoric antibiotics such as monensin and related compounds have been the single most successful class of rumen manipulators to reduce ruminal protein breakdown and to decrease methane production in rumen fluid. However legislation (1831/2003; EC, 2003) was introduced within the European Union to prohibit the use of growth-promoting antibiotics, including monensin and related compounds, in animal feeds. Given the general level of consumer concern over additive use in feeds it is unlikely that chemical inhibitors of protein degradation and/or methane formation are likely to find favour in the market and thus a number of screening projects in recent years have investigated exploiting natural products or processes as alternative rumen manipulators. The information on the effect of feed additives and dietary strategies will be generated in this project to help to reduce ruminant greenhouse gas emissions. |
| 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)? | Currently animal agriculture is perceived as inefficient and wasteful in terms of land and nutrient use, this programme of work will improve the efficiency of animal production systems and the quality of the final product. The outputs of this project are targeted and relevant at various levels of the food chain from the farmer producer, to the meat processor, the retailer and the consumer. In particular, the improved ability to (1) feed ruminants under high forage input systems or sustainable protein systems (2) predict nutrient supply and (3) to manipulate production response will lead to improvements in agricultural efficiency both in terms of use of natural resources and in farm profitability. This will contribute to a reduced reliance on imported feeds, particularly imported protein |

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| | sources, which is especially prudent following the recent and on-going problems in our industry. Improved knowledge of the interrelationships between growth of an animal and subsequent effects on eating quality (for example tenderness) will improve the quality of the product, which is of benefit to the producer and the consumer. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Over the course of the project it is envisaged that approximately 600 sheep and 300 cattle will be used in growth and efficiency trials. |
| 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 experience little more than standard farming procedures. All procedures will not exceed mild in severity (e.g. blood sampling and faecal grab sampling). At the end of the trial animals will either be returned to the form or sent off for commercial slaughter. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | For some of this work there is a requirement to measure various combinations of feed intake, rumen fermentation parameters, product quality, and mineral (particularly N) partitioning between productive (growth and product) and excretion (urine and faeces) purposes. These data can only be collected from live animals, and for the purposes of measuring parameters in relation to meat production there is a clear need to use cattle and sheep. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | We operated standard quality assurance procedures (i.e. the BBSRC/Defra/FSA Joint Code of Practice). Statistical advice is sought (from North Wyke statisticians) on all experimental protocols to ensure that maximum information are obtained from the minimum resource. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the | For much of the work to be carried out the effects of treatments on feed intake, production, composition, and/or nitrogen and methane excretion in/from specific animal groups (e.g. sheep or cattle) is required. Therefore, these animals are most suitable |

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| objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | and refined for use in this work, decisions on number of animals will be driven by the statistical design as discussed previously.<br><br>All animals will be daily assessed for health and well-being, as determined by alertness, feed and water intake. Any sign of ill health will be reported to the Veterinary surgeon with the animal being removed from trial if symptoms persist or are greater than mild in severity. |

| **Project 2** | **Determining red fox movement patterns around breeding birds of conservation concern** | |
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| Key Words (max. 5 words) | Fox, tagging, collar, capture, conservation | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | | Basic research |
| | | Translational and applied research |
| | | Regulatory use and routine production |
| | Y | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | Y | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | **Summary of project objectives**<br><br>To determine the movement behaviour of foxes around breeding wading birds. | |
| 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)? | By identifying suitable management strategies, we aim to reduce predation losses and improve productivity of declining wader populations. This will help substantially to avert what at present appears to be an unavoidable loss of biodiversity. | |
| What species and approximate numbers of animals do you expect to use over what period of time? | Red fox *Vulpes vulpes*<br><br>Initially up to 10 animals before review of procedures and sample size, perhaps extending to 50 over 5 years. | |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected | No lasting adverse effects are expected for foxes as a result of capture or tagging.<br><br>However, we have considered the risks of | |

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| level of severity? What will happen to the animals at the end? | unexpected outcomes leading to poor welfare, particularly for non-target species captured in the same devices, and have planned how to deal with these in an appropriately humane manner. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The purpose is to study the movement behaviour of wild foxes in relation to wading bird species of conservation concern, breeding in representative habitats. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | The number caught and tagged will initially be constrained to a maximum of 10. This will be reviewed once we have initial result from which we can calculate appropriate sample size, allowing for individual variation and tag loss or malfunction. |
| **3. Refinement**<br><br>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 capture, tagging and handling methods are of course intended not to cause any lasting harms, otherwise the work would be meaningless. However, a non-zero risk of adverse effects occurring must be acknowledged. Our capture protocol, developed over 3 decades, was intended – and has been shown to – reduce such risks to tolerable levels. The use of radio-collars also carries some acknowledged risk, however we are very experienced with the species, and believe we can judge suitable fit as well as anyone. Radio-tagging has the advantage that the well-being of animals can be monitored while tags remain active. |

<table>
<tr><td>Project 3</td><td>Acoustic Fish Tracking in Tidal Waters</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Acoustic tracking, Salmon, Trout, Eel</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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</td><td>~~Yes~~</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>It is proposed to track migratory fish as they emigrate from, and return to freshwater systems that may be affected by the construction of marine renewable energy schemes; also, to track marine fish that frequent inshore areas and may be affected by the construction, operation and decommissioning of such schemes. The aim is to determine basic fish behaviour such as swimming speed, bearings and track tortuosity in free-swimming fish. The data will be used to calibrate an existing model that predicts the frequency at which fish will encounter turbines within such schemes in which they may be injured or killed. The model will inform the design and location of the schemes so as to reduce the impact of the developments on fish populations.</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 collection of empirical data relating to the near shore and coastal movements of migratory and marine fish, as proposed, is designed to support the development of a model to improve the assessment of interactions between migratory and marine fish and coastal renewable energy developments. The development of an evidence based species- and lifestages-specific model will improve regulators' confidence in impact assessments and in turn will provide a greatly enhanced EIA tool for future marine renewable energy projects.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The project proposes to tag a range of species including Atlantic salmon (Salmo salar), sea trout (S. trutta) and European eel (Anguilla anguilla) bass (Dicentrarchus labrax), rays (Raja spp.) and whiting (Merlangius merlangus) and a number of location</td></tr>
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| | around the UK coast. It is anticipated that approximately 750 fish will be tagged over the life 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 external attachment of a tag weighing no more than 2% of the mass of the fish for a relatively short period of its migration is believed to have a minimal negative impact on the fish in the study. The attachment procedure is considered to be of a minor severity. The fish will be maintained under general anaesthesia throughout the tag attachment procedure.<br><br>If pre-tracking trials indicate significant detrimental impact arising from external tagging we shall revert to internal tagging methodology requiring a surgical procedure. All surgical procedures have the potential to result in health problems for fish such as disturbance of physiological function, or more subtle behavioural or immunological effects. However following a short period of perturbation following capture and tagging no significant effects on swimming behaviour are typically observed in fish following this procedure.<br>Handling stress will be improved by rendering the fish unconscious (anaesthesia) during the operating procedure. The procedure to attach the transmitter to the fish is considered to be at a moderate level of severity. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Live fish are needed within the study to bound and calibrate behavioural rule sets for particles (virtual fish) within the model. The use of wild fish is preferable because the swimming capabilities and behaviour of hatchery-origin stock may be biased by the condition, and learned behaviour of captive fish. The validity of results based on non-wild fish may be challenged. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | The sample population studied will not be of a uniform composition. Sex, age, length, weight and health will vary, and may affect behaviour and swimming ability. It is important that a sufficient sample is tested to represent these characteristics within a population. |
| **3. Refinement**<br>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 | The fish species chosen are species of conservation concern and the study will inform management and regulatory objectives designed to derive long-term conservation gains for these species.<br><br>Were appropriate to do so fish will be tagged with |

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| minimise welfare costs (harms) to the animals. | external tags that are both quicker and less invasive to fit, potentially reducing the initial stress levels to the fish. The behaviour of fish within each trial will be observed to determine whether this method of tag attachment is fit for purpose or whether a method of internal attachment is required. Whilst initially more invasive, surgical implantation to the peritoneal cavity represents a secure form of attachment from which individuals have been shown to recover rapidly with no lasting harm. This method is however considered to be of moderate severity.<br><br>The use of an anaesthetic to sedate the fish during transmitter attachment offers effective pain control. The wound site is to be treated topically with a povidone-iodine disinfectant to provide biological protection.<br><br>Minimal fish handling and the employment of fish husbandry welfare techniques will ensure minimal stress to the fish prior to and following the surgical procedure. Fish recovering from anaesthesia will be observed in a dark environment with minimal disturbance. |

| **Project 4** | **Ecology of small carnivores and their prey** | |
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| Key Words (max. 5 words) | Wildlife conservation, predator-prey, ecology | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | | Basic research |
| | | Translational and applied research |
| | | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | X | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Predators, such as pine martens and polecats, are recovering their populations after historical population control and habitat loss. While recovery is proceeding rapidly for some species in some parts of Great Britain (e.g. pine martens in Scotland, polecats in Wales), in other areas their populations remain relict (e.g pine martens in Wales). This project will improve understanding of predator population recovery and translocation as a conservation tool. The project will also help understand the direct and indirect impacts that predators have on their prey. | |
| 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)? | An investigation of translocated animals will allow us to test assumptions of habitat suitability and to refine the assessment process and release protocol for future translocations. Many translocations have failed to restore self-sustaining populations, however there is a significant lack of information available from past translocations to inform future efforts. This study will contribute to the body of scientific work on conservation translocations and on the movement | |

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| | and space-use of predators and their interactions with other species. Another benefit will be in helping to understand how populations and behaviour of prey, including non-native “pest” species, such as grey squirrels, might be affected by recovering predator populations and to assess the potential for predator restoration to contribute to pest management at a larger scale. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Around 500 carnivores (pine martens and polecats) and around 1400 individuals of their prey species (rodents, rabbits and shrews), between 2015 and 2020 |
| 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? | These are all minor procedures with a very low risk of any adverse effects. Minor abrasions from traps are somewhat likely in some larger species. Abrasions from collars are unlikely. Animals will be released back into the wild, under licence from the statutory nature conservation bodies where necessary. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The project is focussed around a study of mammals in the wild. Therefore it is not possible to conduct this work without the use of animals, and cannot be predicted through analysis of existing information or simulation. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Modelling has been used to estimate the minimum number of animals required for the translocation. Our sample sizes are based on this number allowing for the additional capture of individuals that are unsuitable for translocation. Prey species sample sizes are based on estimates of variation between individuals and sites. |
| **3. Refinement**<br><br>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 | The studies aim to gather species-specific information in order to inform species conservation.<br><br>Our methods, including biotelemetry, genetic and stable isotope analysis, have been used to good effect with many species of mammal including mustelids and rodents, in order to gain valuable information on home range, habitat use and |

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| minimise welfare costs (harms) to the animals. | dispersal. Collars will never be over 10% of the weight of the animal. Every effort will be made to recapture the animals in order to remove the collar. |

| **Project 5** | **Detection of *C. botulinum* spores/toxin** | |
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| Key Words (max. 5 words) | Clostridium, botulinum, toxin, spores, bioassay | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | N | Basic research |
| | Y | Translational and applied research |
| | N | Regulatory use and routine production |
| | Y | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | N | Preservation of species |
| | N | Higher education or training |
| | N | Forensic enquiries |
| | N | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Food producers are obliged to ensure the microbiological safety of all food supplied for human consumption (Food Safety Act, 2001). Food contamination by *Clostridium botulinum* can have severe and possibly fatal effects on consumers (man and animals) and is an important consideration in production and processing of foods and feed. As *C. botulinum* is ubiquitous in the environment, detection of low level contamination, particularly in foods destined for vulnerable groups of the population (e.g. infants) is an occasional but important need. Contamination may also occur post process or as a result of changed formulations or processes. In the UK hazelnut yoghurt outbreak in 1989, 27 people were affected when a change in formulation occurred without an appropriate change in processing. The oral lethal dose for an adult human is very low (5ng) which means a very sensitive test is required when an outbreak is suspected. The MLA is the most sensitive and appropriate assay available with current technology. The objectives of this project are to *1.* | |

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| | Examine food samples suspected of containing botulinal toxin (following a processing failure or suspect botulism contamination). 2. To challenge test new products or changed processes/formulations with *C. botulinum* to determine if growth/toxin production could occur. 3. To examine clinical isolates/suspect cultures for the presence of botulinal toxin. 4. To examine clinical specimens from Veterinary establishments in support of clinical diagnoses. 5. To examine feeds (consumed by food producing animals) for the presence of *C. botulinum* spores/toxin. |
| 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)? | Benefits are reducing the risk of food borne botulism and protecting consumers. Information as to whether the growth/toxin production *of C. botulinum* would be possible within new formulation/process change would be provided to enhance protective measures. Examining products suspected of causing botulism or clinical isolates will help identify the source of contamination and enable rapid treatment and remedial actions. Advancing analytical technology is closing the gap between in vitro and in vivo methods. Should sensitivities indicate a possible move to in vitro models during the licence period, some mouse lethality assays (MLA) may be required to finalise method validation to ultimately replace the animal based assay. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Mice, young adult, up to 250. Only 38 samples have been tested since 2005. Large numbers are only used in the event of an outbreak situation. Four mice are used per sample. |
| 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? | After intra-peritoneal injection of the sample extract mice are subdued due to the injection for approximately 15-30 minutes after which they recover fully.<br><br>Confirmed signs of botulinal toxin intoxication include pinching of the flanks, mice will be subdued, laboured breathing) and terminal stretching of the fore and hind legs. Mice are observed regularly (hourly) for the first 24 hours and then checked 4 times a day after that for the duration of the test (3 days). From experience, |

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| | if signs do occur then they will normally appear in first 24 hours, If preliminary signs of botulism are observed (slight pinching of waist, rapid breathing) checks are increased to every 20-30 minutes until confirmed signs or botulism are observed (increased pinchinq of waist, cheyne stokes respiration, laboured breathing) at which point animals are killed humanely at once.<br><br>At the end of test (3 days) all remaining mice are killed humanely.<br><br>Some mice (controls and spikes) may experience a severity level of severe through exhibiting typical signs of botulism, however the majority will only experience a mild severity level as relatively few samples are found to be positive.<br>The mice are checked often enough to ensure none die from botulism but are humanely killed as soon as typical symptoms are determined. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Alternative in vitro detection assays i.e. immunoassays, endopeptidase assays, mass spectrometry and cell based assays have significantly progressed over the last 10 years although no single method has emerged that can detect all botulinum neurotoxins in food at levels similar to those achieved by the MLA. Most alternative methods do not detect all toxin types and many do not detect active toxin. Low levels of toxin may not be detected by in vitro screening techniques but will be positive with the MLA. This is particularly important when you consider the oral lethal dose for an adult human is as low as 5 ng (5000 pg) of toxin for some strains of *C. botulinum.* The mortality rate for botulism is still 5-10% despite modern therapy which is still high for a food borne illness. Rapid treatment with antitoxin is an important factor in reducing the fatalities and severity of illness, so rapid accurate sensitive tests are of paramount importance |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers | The current method in use for the detection of *C. botulinum* or its toxins is the mouse bioassay. The number of animals used is the minimum number of animals required by this published method (4 per |

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| of animals | assay).<br><br>Toxin typing is only carried out if required following a positive result. It is never undertaken without the confirmed presence of toxin present.<br><br>Where possible if multiple samples are to be tested these are pooled and only tested separately if a positive result on the pooled sample is obtained.<br><br>We do not quantitate toxin levels thus further limiting the number of mice used.<br><br>Ethical approval is sought prior to any testing to ensure that no unnecessary testing is carried out. |
| **3. Refinement**<br><br>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 mouse bioassay is a regulated, recognised published method for the detection of botulinal toxin and details the choice of species to be used. This method uses the minimum number of animals to give the results required to determine the presence of botulinal toxin.<br><br>Animals are observed regularly (hourly for the first 24 hours and then 4 times/day for remaining test) for typical signs of botulinum. If preliminary signs of botulism are observed the frequency of checks is increased (to every 20-30 mins) until confirmation of botulism (usually a maximum of 2 hours). Once confirmed signs are observed, animals are killed immediately by a humane method. Experience has shown that if symptoms do not occur during the first 24 hours they are unlikely to occur. |

<table>
<tr><td>Project 6</td><td>Chemicals and fish research</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Toxicity testing, environmental pollutants</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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></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><u>Yes</u></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</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>1 To determine the chronic toxicity of chemicals or formulations of chemicals intended for use in the aquatic environment or that may reach the aquatic environment as a result of application within a process, accidental spillage or malicious action.<br>2 To determine the quality of waterbodies contaminated by human activities to sustain wildlife and/or human health and to evaluate the benefits of different treatment options</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 key benefit is for protection of the environment from the detrimental effects of an increase in the concentration of chemicals. These chemicals may have the potential to impact on aquatic species and human health as a consequence of contact or ingestion of the water or of aquatic organisms that have been in contact with the water</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Over the five year study period it is possible that a number of different fish species may be tested either as standard laboratory species or according</td></tr>
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| over what period of time? | to their relevance to a particular pollution issue that is being investigated. Up to four and a half thousand could be tested over this period with some of these sourced from the wild. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Procedures where death of the test fish is possible will be conducted so as to minimise the number of fish used and observations will be conducted sufficiently frequently that fish can be killed humanely as soon as they become moribund. For other tests the effects may be weight change or change in growth, fertility or behaviour. At the end of the tests fish will be humanely killed |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The purpose of the work is to record the impact of chemicals used in the aquatic environment to manage pest species or to treat chemical or oil spills. It is important to confirm safe working thresholds for chemicals to enable effective use and to safeguard fish both at the individual and population level. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | All experimental work will utilise the published literature and previous experience by the Project Licence holder and colleagues who undertake similar work to ensure that the minimum number of animals are used that will permit a robust statistical and meaningful analyses of the results. |
| **3. Refinement**<br><br>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 purpose of the work is to provide advice on the conservation and management of fish stocks. Therefore, a range of fish species are likely to be used to reflect sensitivity of natural populations. The methods chosen are based on previous experience and research and will provide evidence that will form the basis of suitable advice to Government and industry on the factors affecting fish populations and recommendations for suitable mitigation. Where fish undergo a procedure and recovery, they will be monitored for a suitable period of time in order to assess any adverse impacts and ensure a minimum of suffering. |

| **Project 7** | **Assessment of medicines for the treatment of external parasites of farmed fish** | |
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| Key Words (max. 5 words) | Salmon, Sea bass, parasites, medicines | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | | Basic research |
| | X | Translational and applied research |
| | X | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Most fish species, as in terrestrial animals, are subject naturally to a range of diseases and parasites. Where fish are farmed fish are held at high density, infections can increase rapidly. Fish farmers therefore treat and protect their stock with licensed medicines. The range of medicines is limited and parasites may build up a resistance to the use of the medicines. The purpose of the proposed work is to test the efficacy and safety of a range of promising new chemotherapeutants for the treatment of a range of ectoparasites mainly Amoebic gill disease which causes gill and respiratory problems in fish, sea lice a major parasite of farmed fish, and also a range of other smaller ectoparasites. To ensure that these treatments are safe, the dosage and duration of treatments would be examined. | |
| What are the potential benefits likely to derive from this project (how science could be | The project is aimed at improving the health and survival of farmed fish. Amoebic gill disease has been present in fish on most fish farms in Scotland in 2012 | |

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| advanced or humans or animals could benefit from the project)? | and has caused up to 5000 tonnes of mortalities, and also caused irreversible gill damage and health problems for the fish, with poor growth and condition. An effective treatment would result in less health and welfare issues with faming salmon.<br><br>Sea lice can cause mortalities in farmed salmon if untreated or if the treatments are ineffective. The estimated loss in fish and production in Europe is 300 million Euros. Sea lice from farms may also impact on wild fish stocks if untreated. There are therefore large welfare, ethical and economic benefits in developing successful alternative treatments.<br><br>The other external parasites that may be found on farmed fish have less economic impact but may cause health issues for stock including skin and gill damage and remedial treatments will therefore benefit fish welfare. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Salmon is the main fish reared in the UK in seawater and *Salmo salar* would therefore be the main species utilised in trials. Wrasse, Lumpfish, Sea bass and, to a lesser extent Tilapia, would also be examined.<br><br>A total of up to 4000 fish over a 5 year period. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | In the case of parasite transmissions, expected adverse effects are likely to reflect the normal host responses to the parasite. This would include increased flashing, increased mucus production, skin and gill irritation and /or superficial damage. Likely level of severity will be mild to moderate and it is expected that based on best practice, this will more frequently be mild.<br>As described previously, a number of adverse effects may be noted during exposure of fish to chemotherapeutants. This includes, but is not limited to loss of equilibrium, changes in locomotor activity, changes in schooling and social behaviour, alterations in body movement and/or colouration and ventilator patterns. Likely level of severity will be mild to moderate and it is expected that based on best practice, this will more frequently be mild.<br>At the end of each study fish will be euthanized by a |

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| | schedule 1 method. Tissue samples will be taken where practicable and used to provide as much information for the study. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | While some proposed medicines can initially be screened with sea lice other parasites off the host in a container for example, the therapeutic effect on the parasite may be less when the parasite is on the host. Other parasites may be difficult to maintain in a viable condition when not on the host and infection and treatment of host tissue in fish is therefore necessary. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | While the minimum number of animals would be used the numbers required are dependent on sufficient numbers for statistical tests to assess whether the treatments have been successful. Also, the experiments would have to be carried out in replicates to ensure that the results are consistent and reproducible. We will base our studies on published guidelines and / or peer-reviewed publications to justify the numbers of fish used. In addition, we will conduct statistical studies on the study design to ensure it is valid. |
| **3. Refinement**<br><br>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 most common fish species farmed globally is salmon and is therefore the most desired experimental animal. Sea bass is also a species of choice as it is the most commonly farmed fish in the Mediterranean. The animal infection models are based on established and tested infection routes enabling an appropriate level of infection and the availability of providing effective outcomes and outputs in the trials. The challenge level of the parasite in the experiments would be pitched at a level to avoid causing undue stress and to permit the viability of the animal until the end of the trial. Fish would be monitored. |

| **Project 8** | **Assessing the risks to fish of environmental stressors** | |
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| Key Words (max. 5 words) | Fish, risk assessment, chemicals, environmental stressors. | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | | Basic research |
| | | Translational and applied research |
| | | Regulatory use and routine production |
| | Yes | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Our rivers contain a myriad of chemicals originating from the activities and lifestyle of man. Relatively little is known about whether or not the concentrations of these chemicals in rivers are high enough to cause negative effects in wild fish. The main objective of this project is to find out if chemicals, either singularly or in combination (mixtures), do pose a threat to wild 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)? | If we can identify chemicals that do pose a threat to fish, then strategies can be put in place to reduce exposure to these chemicals, thereby improving fish welfare in our rivers. If results show that mixtures of chemicals can also pose a threat even if the individual chemicals do not, these would increase the pressure for environmental legalisation to encompass mixtures toxicity. The overall benefit of the project will be cleaner rivers that are better able to support healthy populations of fish (and other aquatic | |

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| | species). |
| What species and approximate numbers of animals do you expect to use over what period of time? | The research will use small native and non-native fish species which are widely used in ecotoxicology research worldwide. The species we used are; Fathead minnow, Zebrafish, Medaka, Sheepshead minnow and stickleback. It is anticipated that most of the research will be conducted with fathead minnows and that around 600 fish will be used each year. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | The majority of studies will be conducted with chemicals known to be entering our rivers. These will be tested either on their own or as mixtures or in combinations with predicted environmental stressors (such as those likely to occur due to climate change). Experiments will be designed to produce subtle effects, for example, some chemicals might affect the ability of the fish to breed, but otherwise have no obvious effects, whereas others might affect swimming behaviour of the fish. We may wish to work with chemicals or stressors whose chronic effect is not fully characterised. Occasionally we may wish to perform pilot studies where the adverse effects may rarely reach the moderate level. We consider it extremely unlikely that any of the research undertaken during this project will cause severe effects. At the end of an experiment, all fish will be killed by an overdose of anaesthesia. No fish will be reused in subsequent experiments. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Laboratory fish need to be used because currently our knowledge is not good enough to enable us to predict the effects of chemicals and other environmental stressors, or combinations of them, with a high degree of confidence. If fish are not used in this type of research project, there is a likelihood that chemicals and other factors that pose a threat to the health of fish will go undetected, leading to undesirable adverse effects on wild fish. |
| **2. Reduction**<br><br>Explain how you will assure | All experiments will be well planned and very carefully designed in order that the minimum number of fish are used to obtain useful robust, repeatable |

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| the use of minimum numbers of animals | data. Non animals (i.e. using computer models or cells in petri dishes) approaches will be used to aid the design of the fish experiments. The number of fish experiments will be kept to the absolute minimum required to get robust results needed to inform governments and policy makers. Advice on experimental design will be provided statisticians, to ensure good quality data are always obtained from experiments involving fish. |
| **3. Refinement**<br><br>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. | Most of the research will be done using the fish species most widely used in ecotoxicology research. This enables all the knowledge about the species (such as their genetic, biochemical, and physiological processes) to be used to maximum effect. Building on an established, large body of knowledge is the most efficient way to proceed. |

| **Project 9** | **Lungworm Vaccine Primary production** | |
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| Key Words (max. 5 words) | *Dictyocaulus viviparus* | |
| Expected duration of the project (yrs) | Five | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | | Basic research |
| | | Translational and applied research |
| | X | Regulatory use and routine production |
| | | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | This application is for a licence to produce a vaccine for use in the control of a devastating and potentially fatal parasitic disease, primarily of cattle, in temperate climates with summer rainfall. The adult parasites of *Dictyocaulus viviparus* are found in the lungs and cause bronchopneumonia, which can be severe and fatal. The parasite is susceptible to modern anthelmintics (drugs which kill internal parasites), but control of the disease by this method is very unlikely to allow natural immunity to develop, thus leaving animals which are susceptible to the infection once the anthelmintic is no longer present within the body. On the other hand, strategic vaccination stimulates development of immunity to the parasite before the calf is put out to pasture, and is a well proven method of control for this disease. This vaccine has an excellent track record spanning over 50 years, so is well proven in the field. Although research is ongoing into finding ways of producing an effective vaccine without using calves, no-one has yet been successful, so we continue with the tried and | |

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| | tested method. We have a very experienced team to manage and care for the calves and to produce the vaccine, and have been very successful in both considerably reducing the number of calves required for the final production process and in minimising the clinical effects of the parasite in our production calves. |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | This vaccine is tried and tested and shown to be efficacious in aiding control of a parasitic disease of cattle which is potentially lethal and has huge welfare implications if left untreated.<br><br>Perceived benefits include:<br><br>• Improved cattle welfare – both milk and beef cattle<br>• Improved cattle production – both milk and beef cattle<br>• Protection of environment through reduced use of anthelmintics |
| What species and approximate numbers of animals do you expect to use over what period of time? | Calves 250<br><br>Guinea Pigs 30<br><br>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? | 1. To rear high quality Specified Pathogen Free calves -including taking a series of blood and faecal samples to check for specified pathogens – no adverse effects are expected except from natural, non-related causes which could occur anywhere.<br>2. To infect some of these calves with Dictyocaulus viviparus (Dv) infective larvae (L3), allow it to complete its life cycle within the natural host and collect larvae in order to make a vaccine against the disease. Calves will suffer symptoms of infection with Dv. which produces a parasitic bronchopneumonia. These symptoms include increased respiratory rate and effort but can be considerably reduced by appropriate treatment. Where treatment fails to adequately control the symptoms, the calf will be euthanased to minimise suffering. Less than 2% are expected to be classified as 'severe'. |
| **Application of the 3Rs** | |
| **1. Replacement** | Although alternatives methods of producing an effective vaccine have been investigated, to date, |

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| State why you need to use animals and why you cannot use non-animal alternatives | none have been successful, so there is no alternative to using calves. 30 to 40 calves can produce enough larvae for over 1 million doses of vaccine. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Around 50 years of production of this vaccine have shown it is very efficacious and also the number of calves used for production has been dramatically reduced since its inception. Experience allows us to use the minimum number of calves to produce sufficient larvae for vaccine and ancillary requirements. |
| **3. Refinement**<br><br>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. | Calves (cattle) are the natural host of the parasite. There is no suitable alternative model to use for larval production.<br><br>Measures to minimise adverse effects on welfare include:<br><br>• Use of effective vaccines to protect against other bovine respiratory pathogens which exacerbate the effects of *Dv* infection.<br>• We are currently designing new calf pens which will give the calves more space whilst still allowing stress free handling for management and treatment (which is vital for calf welfare). These should be in place by the time any calves are used for production on this licence. |

| **Project 10** | **Studies of virulence in human and animal fungal infections** | |
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| Key Words (max. 5 words) | fungi, virulence, infection | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | ✓ | Basic research |
| | ✓ | Translational and applied research |
| | | Regulatory use and routine production |
| | ✓ | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | ✓ | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The overall scientific objective of the project is to determine the reasons for which fungi are able to cause infections in a wide range of vertebrate species, both wildlife and human. Specific objectives will identify the classes of genes that determine fungal virulence, and the rates at which genomes are able to adapt and to infect novel hosts, as well as to evolve resistance to antifungal therapies. | |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | The research seeks to establish methods by which we can combat the increasing incidence of fungal infections in both natural and human-systems. We will do this by developing methods to mitigate infection in natural populations, by translating our findings into novel diagnostics and/or by developing antifungal therapies. | |
| What species and approximate numbers of animals do you expect to use | Amphibians: Anurans - Common toad (*Bufo bufo*), common frog (*Rana temporaria*), midwive toads (*Alytes sp.*), Golden Mantella (*Mantella aurantiaca*), | |

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| over what period of time? | clawed frog (*Xenopus tropicalis*).<br><br>Caudates - Common newt (*Lissotriton vulgaris*), Palmate newt (*Lissotriton helveticus*), Great crested newt (*Trituris cristatus*), alpine newt (*Mesotriton alpestris*), fire salamander (*Salamandra salamandra*), oriental newt (*Cynops orientalis*).<br><br>In total, we will use up to 11,510 amphibians. Of these, 2,610 will be entered into protocol 1 (Toe clipping) and up to 8,890 will be entered into protocol 2 (Exposure to fungus). These animals will be used across the five-year period of the licence.<br><br>Mice: We will enter up to 2,000 animals into protocol 3 (Analysis of virulence). These will be used across the five-year period of the 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? | **Amphibians:** The animals may be toe-clipped under local anaesthesia for a single hind-digit then released back to into their environment once satisfied they are in a fit and healthy state to do so. Animals may be inoculated with chytrid fungi. This will lead to the establishment of infections which may cause death without preceding clinical signs in around 10-25% of juvenile amphibians. Humane end points to reduce severity include loss of the ability to turn over (loss of righting reflex), excessive lethargy and weight loss. At the end of the experiments the animals will be humanely culled.<br><br>**Mice:** The mice will receive immunosuppressive drugs and then be infected with fungi. This will lead to establishment of infections, which may cause the animals to become distressed. At the end of the experiments the animals will be humanely culled. Suffering will be minimised as all infections will be undertaken under general anaesthesia and in addition 20% weight loss has been established as a surrogate marker for death previously (and which point mice will be humanely culled). |
| **Application of the 3Rs** | |
| **1. Replacement** | Our research needs to be relevant to the species that we are trying to conserve. This context-dependence |

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| State why you need to use animals and why you cannot use non-animal alternatives | means that non-animal alternatives will not yield the answers that we need.<br><br>**Mice:** Fungal infections in immunocompromised humans are complex and involve a number of host and pathogen factors that cannot be modelled in simple cell-culture assays. There are complex interactions that occur at a whole organism and inter-organ level that need to be understood in order to develop better diagnostic and therapeutic approaches to these life threatening infections. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | In all instances the minimum number of animals will be used to enable statistical significance. In addition we are developing non-vertebrate models of infection that will enable further reduction of the number of animals used. |
| **3. Refinement**<br><br>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. | **Amphibian:** We carefully choose our species based on field data showing which species are being most severely affected by the emerging infections that we study. These species are the most conservation-relevant and therefore are our focus in the laboratory. All animals will be housed in aquaria or vivaria with appropriate habitat enrichment. Animals that reach experimental end-points will be humanely culled.<br><br>**Mouse:** Murine models of infection offer several distinct advantages for studying invasive fungal disease, including ease of use, reproducibility and availability of immunosuppression regimes which mimic host factors of human disease. Additionally murine models offer the opportunity to investigate evolution of drug resistance through the serial passage of fungi in animals receiving drug-therapy, mimicing population-level attributes human infection. Suffering will be minimised as all infections will be undertaken under anaesthesia and in addition 20% weight loss has been established as a surrogate marker for death previously (and which point mice will be humanely culled). All animals will be housed in groups with appropriate environmental enrichment and fed according to current 'best practice' at Imperial College Central Biological Services (CBS) |

| **Project 11** | **Exposure of animals to animal viruses for diagnosis and characterisation** | |
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| Key Words (max. 5 words) | Animal, Viruses, Diagnosis, Characterisation | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | X | Basic research |
| | X | Translational and applied research |
| | X | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The objective of this project is to assess new viruses which may infect and cause disease in UK livestock breeds. This will help the UK government establish transmission mechanisms and develop control and preventative measures, diagnostic tools and isolate the causative agent. | |
| 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 described in this licence will help with the early and accurate diagnosis of viral diseases of livestock. Enabling outbreaks and incursions to be prevented or significantly curtailed. This has a major welfare benefit to the animals which would otherwise have become infected. This work is very beneficial to UK livestock and may help prevent thousands of animals getting infected with obvious welfare benefits. It may also help prevent huge financial losses to the UK agricultural sector. | |

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| What species and approximate numbers of animals do you expect to use over what period of time? | Cattle —30 Sheep — 30 Goats — 30<br><br>Embryonated hens eggs (10-11 days old) — 100<br><br>Neonatal mice (1-2 days old) — 100,<br><br>lactating females-30 |
| 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 some of the protocols the host species need to be infected with a virus that may give them clinical signs of that disease. At the end of the experiment full use of the animals will be made as animals will be bled out to collect the maximum amount of blood to produce diagnostic reagents. Embryonated eggs and mice are also used to isolate these animal viruses if cells cannot be used to diagnose or isolate the virus and are humanely killed at the end of the experiment. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | To see the effect of these viruses on UK breeds it is necessary to infect the natural hosts such as cattle and sheep. Susceptible animals are also required to produce diagnostic reagents. Embryonated eggs and neonatal mice may need to be used to diagnose and isolate viruses where isolation in cells has been unsuccessful. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Due to natural variation there are a minimum number of 5 animals which need to be used for the production of antisera and for each infection study. Where appropriate the advice of a biometrician will be sought and statistics used to ensure appropriate groups and numbers and to ensure interpretation of the results is meaningful through experimental design if we are unsure as to the correct group size.<br><br>For some of the proposed work small animal models can be used to reduce the use of large animals and where appropriate cell culture will be used to isolate viruses. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most | The animals chosen include those that are susceptible to the viruses and others are established models used in diagnosis and virus isolation. |

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| refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Extra monitoring is included in the protocols. |

| **Project 12** | **Effects on metabolism and endocrine pharmacology** | |
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| Key Words (max. 5 words) | Metabolism, Endocrine, Safety, Efficacy, Pharmacology | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | | Basic research |
| | X | Translational and applied research |
| | X | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Compounds that interact with the endocrine system are known to produce profound effects in nature and humans even when the individual is exposed to very small doses. Any system in the body controlled by hormones can be detrimentally affected by hormone disruptors. Specifically, endocrine disruptors may be associated with the development of sexual development problems such as feminizing of males or masculinizing effects on females, etc. It is therefore important, where possible to produce drugs and other chemicals that are well tolerated and as free as possible from such side effects. Much of the work conducted under this Licence will be concerned with side effect profiling with the ultimate aim of minimising side effects such as endocrine disruption.<br><br>This Licence also allows for efficacy testing that will, for example, assess potential useful drugs affecting metabolic processes and correcting effects that may occur when metabolic imbalances occur eg in | |

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| | diabetes, high cholesterol and anaemia. Such conditions/disease states often affect large numbers of people. |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | Governments require (and the public expects) that substances we are exposed to are safe or their hazards are well understood. It is an internationally mandated legal requirement. Regulatory approval is required to allow drugs to be tested in human or veterinary trials, or for chemicals, agrochemicals to be marketed. Novel drugs may be developed with reduced or limited metabolic/endocrine side effects or a side effect profile that may be better tolerated than currently marketed products.<br><br>Alternatively, novel drugs that produce beneficial effects via actions on the metabolic/endocrine system may also be assessed and developed as part of this licence. |
| What species and approximate numbers of animals do you expect to use over what period of time? | The species and anticipated usage over the lifetime of the Licence (5 years) are below:<br><br>Rat: 7,300<br><br>Mouse: 2,500<br><br>Guinea pig: 500 |
| 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? | Early studies are performed on the basis of limited information and there may be uncertainty regarding the severity of the response. Most animals are expected to experience no more than mild transient effects such as weight loss or changes in demeanour. A small percentage of animals may show more significant adverse effects indicating moderate severity eg. a very small number of animals may potentially experience severe adverse effects were it not for humane end-points (early intervention or humane euthanasia) to prevent unnecessary suffering.<br><br>Animals in surgical studies may experience some adverse post-operative effects similar to those experienced by human patients, however, supportive treatments are given to eliminate or minimise these |

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| | and appropriate humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with pain relief and/or antibiotic cover provided during and after, as appropriate.<br><br>On study completion, some animals may be re-used in other studies, but most animals are humanely killed using an appropriate method. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Although non-animal (lab bench or computer based) studies can provide useful supporting data to limit and decrease the number animal studies, meaningful and reliable evaluation of whole body exposure can only be comprehensively achieved in studies using intact animals where all the organs and systems are intact, interacting with each other and interacting with the compound, yielding a naturally complex interdependent system.<br><br>For this reason, in vitro and ex-vivo test systems in isolation remain inadequate alone. Use of in-vivo animal models remains a mandatory legal requirement; currently, for many of the study types in this project, there is no scientifically, ethically or legally acceptable non-animal alternative available. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | A logical tiered/sequential approach is generally adopted. Information is reviewed to decide whether testing is appropriate and ethically acceptable and the studies in a program are designed to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals. The numbers of animals used are kept to the minimum commensurate with meeting study objectives and regulatory requirements and further input from statisticians used where appropriate, to ensure robustness and relevance of the scientific data produced.<br><br>Where study designs allow, common controls may be used whereby a number of test substances under investigation may be tested and where comparison against a control is required, a single control may be used against which all the test substance groups may |

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| | be compared thereby reducing the total number of animals required for testing. |
| **3. Refinement**<br><br>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 project uses rats, mice and rarely guinea pigs.<br><br>The animal models described in this Licence are considered to be the most refined as consideration has been given to the methods being the least invasive to the animal whilst maximising the likelihood of generating quality scientific data that will answer the requirements of the piece of work being conducted.<br><br>All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).<br><br>Study designs are reviewed and new methods considered as technology best practice and standards improve and advances become adopted and approved by international regulatory agencies.<br><br>Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering.<br><br>The use of biomarkers has proliferated greatly over recent years and continues to do so and therefore many studies that used to be conducted using disease models have been refined. In such cases, assessment of biomarkers in the blood of animals has replaced the need for the animal to experience the full condition/disease. |

| **Project 13** | **Environmental Toxicity, Metabolism and Fate of Materials** | |
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| Key Words (max. 5 words) | Ecotoxicology Metabolism Fate | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br>(Mark all boxes that apply) | | Basic research |
| | | Translational and applied research |
| | x | Regulatory use and routine production |
| | x | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | To determine the environmental impact of experimental materials as a regulatory requirement. | |
| 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)? | Conducting ecotoxicology studies allows beneficial materials e.g. pharmaceuticals and agro-chemicals to be manufactured and used safely and appropriately for use so as not to harm the environment. | |
| What species and approximate numbers of animals do you expect to use over what period of time? | Rainbow Trout/Bluegill Sunfish < 6000 per 5 Years<br>Fathead Minnow < 10,000 per 5 Years<br>Zebra Fish < 500 per 5 Years<br>Xenopus < 500 per 5 Years<br>Other Fish species < 1000 per 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? | Studies will be conducted under five categories as follows:<br><br>Short Term Acute (Severe)<br>Chronic Reproduction (Severe)<br>Chronic Development (Mild)<br>Prolonged bio-concentration and metabolism (Mild)<br>Prolonged Acute (Severe)<br><br>Upon completion of a test all animals will be euthanized using schedule 1 techniques. | |
| **Application of the 3Rs** | | |
| **1. Replacement** | Regulatory testing requires the use of vertebrate test | |

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| State why you need to use animals and why you cannot use non-animal alternatives | systems, including fish and amphibians, in order to identify any impact or long term effect an experimental product will have on the environment.<br>In order to correctly register a product for use and manufacture it must first be subjected to the relevant testing in accordance with recognised guidelines. Information on aspects of experimental design, including species and numbers of animals required are detailed within these guidelines.<br>Regulatory Authorities have accepted very few non-animal alternative tests, therefore at this time in vivo data are still required. This is expected to be the case throughout the time period of the licence, but any alternative methods that are accepted and suitable will be used should they become available.<br>Where invertebrate use alone is acceptable, such species will be used, in compliance with relevant guidance. . It will be company policy to consult with the DB-ALM, ECVAM, NC3Rs and FRAME websites frequently and research any viable replacements for in vivo testing as part of the AWERB agenda. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Regulatory studies will be conducted in accordance with OECD guidelines where possible. Within these guidelines minimum numbers of animals required to achieve scientific outcomes are detailed and will form the backbone of experimental study plans. The minimum numbers of animals stated within any required guidelines will be used where possible. Any deviations from OECD guidelines will be documented and justified within the study data.<br>Prior to conducting any studies involving protected animals, information on the likely toxicity or possible effects will be requested or researched. If no such information is available dose ranging tests can be performed prior to the main definitive test. A typical dose ranging test would consist of four widely spaced test concentrations and would use a reduced number of animals within each treatment group. This approach allows for the definitive test to be refined, potentially resulting in reduced animal usage and the overall severity of effects.<br>It is often the case, that work involving protected animals will be required alongside a package of studies using for example insects or plants. When this is the case, where possible, studies will be conducted on plants and insects first, in order to identify approximate toxicity and therefore minimise the suffering of protected animals.<br>Recent regulation within the European Union known as Registration, Evaluation, Authorisation and |

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| | restriction of Chemicals (REACH) came into force on the of June 2007, replacing a number of other European Directives and regulations with a single system. This system has various aims in terms of providing protection of human health and the introduction and maintenance of market availability of chemicals. One of the methods it uses to achieve this is the sharing of data. Suitable data held by one company regarding the testing of a material must be shared with other companies, therefore removing the need for additional animal testing, and so reducing overall animal use. |
| **3. Refinement**<br>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. | Protocols 1, 2 and 5 of this project licence are categorised as severe. This is because of a regulatory need to acquire information on acute toxicity and sub-lethal effects of the materials being tested, including the estimated $LC_{50}$, where possible. The use of dose ranging tests and implementation of humane end-points will minimise animal suffering as much as possible. Fish will be added to acute toxicity test systems individually or in small batches to identify any severe reactions/effects a test material may have immediately following exposure. This technique allows scientific staff to justify a valid endpoint without further fish being exposed to the test system and enduring unnecessary suffering. The exact method will be outlined within company standard operating procedures and within study plans (for example, addition of fish at 1 minute intervals followed by close observation prior to the addition of the next fish). An internal policy document on guidance for clinical signs which may be noted as mild, moderate or severe may be used to assist in implementing end-points as required. For Protocol 1 tests, where the aim is to estimate the $LC_{50}$, the ENV/JM/MONO (2010)17 document – Series on Testing and Assessment No. 126: Short Guidance on the Threshold Approach for Acute Fish Toxicity – will be followed as closely as possible in order to determine the information required for regulatory purposes, whilst also minimising suffering and reducing animal use. This document details the use of information from acute toxicity testing (*Daphnia* and algae) to determine a suitable threshold concentration at which to expose fish, in order to determine the species most sensitive to a particular test substance. This approach will also be used for Protocol 5 tests, where possible.<br><br>The selection of species is carefully considered in |

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| | light of the properties and the end use of the test material. The CVMP/VICH/790/03 2004 document discusses how the potential use of the test item aids in species selection for environmental testing. For example, chemicals and biopharmaceuticals intended for use with terrestrial animals should only be tested on freshwater species. Testing of volatile chemicals requires a species which can endure lower oxygen contents and survive static conditions. Chronic studies investigating early life stage development requires robust species which reproduce easily and achieve life stages in a shorter time period therefore reducing exposure periods and suffering. Environmental enrichment will be used where possible whilst holding, culturing and testing animals, there will be particular emphasis on developing appropriate enrichment within test systems in order to minimise stress and yet maintain scientific integrity. This has a mutually beneficial outcome as many observations thought to be related to the test material could indeed be as a result of stress. |

| **Project 14** | **Monoclonal and polyclonal antibody production** | |
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| Key Words (max. 5 words) | Monoclonal polyclonal antibody | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | √ | Basic research |
| | √ | Translational and applied research |
| | | Regulatory use and routine production |
| | √ | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The aim of this project is to raise polyclonal and monoclonal antibodies to a number of diverse targets that pose a threat to agriculture in the UK and overseas**.** The antibodies will be used to develop immunoassays that can identify these targets, including insects, mites, fungi, bacteria and viruses. There is a need for rapid detection and monitoring of these targets and immunoassays offer a quick, simple and cheap method of detection, while also providing great specificity and sensitivity. The antibodies produced will be used in lab-based immunoassays and may also be incorporated into rapid detection kits that are able to provide a result in the field within five minutes with no additional equipment or scientific expertise required and at minimal cost.<br><br>We are also developing a biosensor for passive in field detection of crop pathogens using antibodies. When the biosensor is activated by the presence of the target analyte, a signal is sent indicating the presence of that analyte. The sensors don't require | |

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| | multiple steps to complete and they are passive, that is they can be used as a 'sit and wait' technology, reporting when an analyte becomes present rather than having to perform many tests over time as is the case with many techniques. This would mean that crops could be monitored for appearance of a particular pathogen before disease becomes established enabling rapid and targeted treatment. |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | Many plant pathogens are quarantine listed and pose a serious threat to UK agriculture, and horticulture. The detection of plant pathogens on crops using rapid detection kits is a major advance for field-based inspectors and of significant benefit to the UK's bio security. Rapid detection kits will also be of enormous benefit in developing countries, where plant and animal pathogens can have a hugely detrimental impact on local communities. In many of these areas, access to sophisticated laboratory based diagnostic tools is limited and hence disease control can be extremely difficult. The use of passive biosensors will enable crops to be treated if and only when a pathogen is detected, reducing yield loss and pesticide use. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We have estimated that we could use up to 90 mice and 20 rabbits over 5 years |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | As the animals will be used for antibody production they will be given a series of immunisations with a mixture of adjuvant and target antigen. If the target has the potential to cause infection it will be made inactive before administration. Adjuvant is mixed with the target antigen to slow the release of antigen, as this improves immune response. Rabbits with a good immune response will be bled from the ear after administration of a numbing ointment, blood samples will not exceed the recommended maximum volumes, (of up to 10% circulating blood volume in a single sample with a 2 week recovery and not greater than 15% of circulating blood volume (56 ml/kg) in 28 days) . Mice have a similar series of immunisations |

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| | followed by a small sample of blood (100 µl maximum) being taken from the tail to analyse for immune response. Mice with a good immune response will be killed humanely by a schedule 1 method and the spleen removed to generate immortal cell lines that produce antibody.<br><br>No adverse effects are expected although temporary swelling occasionally occurs at the site of the first injection. All procedures are considered to be mild.<br><br>At the end of projects mice are killed humanely by a schedule 1 method. We aim to re-home rabbits at the end of projects, if this isn't possible they are killed humanely by a schedule 1 method. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Recombinant antibodies (antibody fragments generated from genes inserted into bacteria) and aptamers (short DNA or RNA molecules) can be used in some applications in place of antibodies. However they are not as stable or robust as conventional antibodies and neither function in applications in which our antibodies are utilised. We have investigated both these methods and will continue to do so as further advances in these technologies are made. Molecular diagnostic methods that can be used in the field are becoming cheaper and will increasingly replace potential antibody based field detection methods. New antibodies are only produced when there are no suitable commercially available antibodies available and where molecular diagnostic techniques cannot distinguish between closely related organisms or where there is a need for a cheap quick field based test that cannot be performed using molecular techniques. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | We strive to keep the number of animals we use per project to a minimum. Monoclonal antibody responses are variable therefore 3 mice are immunised to maximise the quality of the antibody produced, this is a reduction from 9 that were initially immunised in the past. If an immune response is poor additional mice would only be immunised if a |

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| | significant change in the protocol has occurred. . For some projects we are able to co-immunise with 2 antigens instead of one which halves the number of animals needed and in two of the projects completed under the current project license we have used fewer animals than were approved for use by our local AWERB. |
| **3. Refinement**<br><br>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 are proposed for polyclonal antibody production because of their known good immunological response. They can be housed and handled easily keeping stress to minimum levels. When immunisations have been completed, blood samples are taken to test for immune response. Rabbits are bled from an ear vein, until recently this was done without pre-treatment but is now done following application of topical anaesthetic.<br><br>Mice are chosen because of their ease of handling and good immune response using established procedures and availability of suitable reagents for subsequent cell culture and assay development. The least invasive lowest volume sampling method will be used to take blood samples, where appropriate anaesthesia will be used and appropriate rest periods will be observed between procedures. Mice have been immunised under anaesthesia but in future this will be done without the use of anaesthesia.<br><br>The severity of all procedures for antibody production is considered to be mild.<br><br>**Total word count section G (NTS): 976** |

| **Project 15** | **The fitness and evolutionary consequences of environmental variation** | |
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| Key Words (max. 5 words) | Selection, environmental change, diversity, speciation | |
| Expected duration of the project (yrs) | 5 yrs | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | x | Basic research |
| | | Translational and applied research |
| | | Regulatory use and routine production |
| | x | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | To determine the fitness consequences for individual animals to changes in the environment.<br><br>To elucidate the evolutionary processes supporting biodiversity and evolutionary change. | |
| 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)? | A better understanding of the evolutionary processes that underpin the development of biodiversity.<br><br>An understanding of how fish respond to environmental change.<br><br>Improved policy for conservation management of particular species in the wild. | |
| What species and approximate numbers of animals do you expect to use over what period of time? | Fish 5500 over 5 years. Approximately 1000 in field studies in the wild and 4500 in the laboratory. | |
| In the context of what you propose to do to the animals, | This project seeks to address fundamental questions about the early stages of the evolutionary process | |

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| what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | and applied questions about how fish respond to human induced environmental change. To achieve this it is necessary to identify individual fish and in some cases their location. Thus this project seeks to anesthetise (mild), mark (mild) or tag fish (mild to moderate), to enable identity and of individual to be determined. The maximum expected level of severity will be moderate but for most procedures it will be mild. Fish will be released to the wild or humanely killed. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The research programme described here addresses questions about ecological, evolutionary and behavioural responses of whole fish to their environment and as a result the objectives cannot be met without conducting field observations and controlled laboratory experiments using fish. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Both field comparisons and laboratory experiments will be designed to minimise the number of fish used. The detailed design of the protocol for any work will aim to maximize statistical power through high quality design which will enable a reduction in the number of fish subjects used. |
| **3. Refinement**<br><br>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. | Freshwater fish, especially those living in post-glacial systems are known to exhibit the characteristics of high levels of within species variation and structuring of form and genetic groups. Thus they offer a good model for testing questions posed in this research programme. The methods and associated protocols reflect the choice of techniques resulting in a modest effect on individual fish. |

| **Project 6** | **Water pollution and salmon magnetoreceptivity** | |
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| Key Words (max. 5 words) | Atlantic salmon, olfaction, magnetoreceptivity, migration, water pollution | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | x | Basic research |
| | x | Translational and applied research |
| | | Regulatory use and routine production |
| | x | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | x | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Atlantic salmon use the earth's magnetic field to guide their migration through their open ocean feeding grounds. They do this using special 'magnetic' cells located in the same part of their brains that process smells in the water. This project will test whether metal-mine water pollution affects the ability of juvenile salmon to detect and respond to magnetic fields. If so, water pollution may interfere with the ability of salmon to navigate their open ocean feeding grounds, thus contributing to population declines | |
| 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)? | Beyond documenting a new scientific phenomenon, this project will determine if water pollution affects Atlantic salmon through disrupting its ability to use magnetic fields to navigate during the migratory phase of its life cycle. If confirmed, the project may initiate a rethinking of regulatory standards for specific pollutants. | |

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| What species and approximate numbers of animals do you expect to use over what period of time? | Atlantic salmon<br><br>Annually approximately 1000-4000 juveniles (approximately 15000 for duration of license) |
| 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? | We do not expect the rearing and testing treatments to have any externally observable adverse effects. We expect animals to experience either 'no or mild severity' effects associated with *olfactory/magnetoreceptivity processes.*<br><br>*All fish will be killed at the end of the experiments using Schedule 1 procedures.* |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | We are testing a hypotheses related to animal (Atlantic salmon, sea/resident trout) migration and this cannot be accomplished without the use of animals. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Our experiment designs are based on the minimum sample sizes (400 fish for each treatment combination) required based on previous research using Pacific salmon. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | We have chosen Atlantic salmon, sea/resident trout because of their variable migratory life histories and previous work on salmonids.<br><br>Animals will be reared at relatively low density in cool, well-oxygenated water using standard fish husbandry techniques. |

| **Project 17** | **Examining dietary specialisations in buzzards** | |
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| Key Words (max. 5 words) | Predation, dietary specialisation, buzzard, isotope | |
| Expected duration of the project (yrs) | 3 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | X | 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 |
| | X | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Individual animals that specialise on a particular food source have been identified in an increasing number of species and populations. These 'dietary specialists', if present, may have broad consequences for population stability, evolutionary dynamics and wildlife management of both predator and prey populations.<br><br>This study will investigate individual variation in the diet of the common buzzard (*Buteo buteo*), a generalist avian predator that holds pair-based territories. This species displays the two prerequisites identified as promoting the evolution of dietary specialisations: weak competition with other species (as they are at the top of the food chain) but strong competition within their own species (due to their high density).<br><br>The question of whether buzzards display dietary specialisations is a particularly pertinent one as there is currently a conflict over buzzard management in the UK due to a perception that some individual | |

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| | buzzards develop a specialisation for hunting released game. As a consequence, licences have been sought to remove specific pairs of buzzards, however, the science to determine whether this is an appropriate strategy ecologically is currently lacking.<br><br>Using stable isotope analysis and prey abundance estimates, we aim to address the question '*do individual buzzards specialise on particular prey species?'* by distinguish the extent to which foraging variation within this species is determined by prey availability or individual differences. Diet and, if present, dietary specialisations, can then be explored against a range of breeding variables (e.g. nesting density, laying date, fledgling number…) to understand the causes and consequences.<br><br>**Aim**: To investigate individual dietary specialisations in the common buzzard (*Buteo buteo*).<br><br>**Objectives**: (1) To examine the diet of breeding buzzards against the prey abundance in each territory to explore if some pairs specialise on specific types of prey. (2) To use estimates of dietary specialisation against breeding variables to look at causes and consequences. For example, how does specialising in a particular prey type influence the breeding success of buzzards? |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | This study will build on the current understanding of foraging behaviour in predators by providing evidence on:<br><br>• Intra-specific variation in the foraging behaviour, specifically in buzzards (see below)<br>• The possible causes of individual dietary specialisations (e.g. population density, prey abundance…).<br>• The consequences of individual dietary specialisations (e.g. on breeding success, chick condition, provisioning rates…)<br><br>The findings of this research will also have species-specific implications as management based on individual foraging specialisms has been repeatedly |

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| | requested for buzzard pairs in the UK. By quantifying the degree to which specific pairs or individuals specialise on certain prey this research will provide evidence to inform the current debate over buzzard management. |
| What species and approximate numbers of animals do you expect to use over what period of time? | This research is on the common buzzard (*Buteo buteo*).<br><br>As this is a new project that depends on the breeding success of buzzards each year, exact numbers are currently unknown. However, we are aiming to monitor between 30-50 nests and a sample a maximum of 200 birds per year (over three 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? | Intravenous blood sampling and taking claw and feather samples are all classified as having mild severity levels.<br><br>Possible complications arising during capture and sampling are:<br><br>1) Blood loss (very unlikely, <1%)<br>2) Impairment to flight or insulation (very unlikely, <1%)<br>3) Stress (very unlikely, <1%)<br>4) Chick explosions, this is when the brood prematurely fly from nest while it is being accessed (unlikely, <5%)<br>5) Injuries during handling or trapping (very unlikely, <1%)<br>Steps will be taken to minimise the chance of any of these occurring and protocol will be in place if they do occur. After sampling, all birds will be immediately released at capture site. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | This work could not be done using computer simulations or other modelling since it requires information from animals living in the wild. For example, the functional response of individual birds to prey availability is not know and so cannot be simulated. |

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| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | As this research is a brief snapshot of buzzard dietary and breeding ecology, it is important to use an adequate sample of nests and individuals. The number of active territories within the study areas is currently unknown but we are aiming to monitor between 30-50 nesting attempts. The number of individuals sampled will be dependent on breeding success. As the variance in the isotopic data is unknown, an a-priori power analysis cannot be undertaken. A post-hoc power analysis will be conducted following the first year's data collection and the sample size refined if possible. |
| **3. Refinement**<br><br>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 common buzzard is an abundant, generalist avian predator, reaching its highest densities in the southwest of England. It displays the two prerequisites identified as promoting the evolution of foraging specialisations (weak interspecific completion and strong intraspecific competition) making it an ideal model species to study this phenomenon.<br><br>Buzzards are diurnal hunters, meaning that the isotope analysis can be supported by direct observations from cameras of prey provisioning.<br><br>This research also addresses a species-specific policy question: do some buzzards specialise on certain prey items? This question is key to a current debate over the management of buzzards in the UK.<br><br>Buzzards will be captured, via methods approved by the British Trust for Ornithology, either as chicks or adults.<br><br>• Captured birds will be immediately be sampled at the capture location to minimise stress to the birds. This also allows them to be released quickly into a familiar environment.<br>• Samples will be taken of claw, feather and blood.<br>Claw: Less than 5mg (total) of tissue will be shaved once a year from several claws to ensure that no talons are blunted or weakened. |

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| | Feathers: will only be sampled once per year, and will be dependent on the age and development of the chick.<br><br>Blood: will be taken a maximum of twice per year for adults and three times for fledglings (one in the nest twice after). No individual will be re-sampled within 3 weeks of previous sampling.<br><br>• Individuals will be identified via colour rings (fitted during initial capture). This will prevent birds from being unintentionally resampled and will allow us to target individuals for recapture if required. |

| **Project 18** | **Ecology of dormice** | |
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| Key Words (max. 5 words) | Hazel Dormice, habitat management, radiotracking, forestry | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | X | Basic research |
| | | Translational and applied research |
| | | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | X | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | There is evidence that the abundance of dormice have declined in Britain and their range appears to have contracted dramatically southwards. In reflection of this threatened status, hazel dormice are afforded protection under the European Habitats Directive (1992) and the UK Habitats regulations (1994). It has been suggested that habitat loss, fragmentation and deteriorating quality have played a part in the decline of the Hazel Dormouse in Britain. Therefore an understanding of the requirements of these animals and the effects of habitat management is needed to try to slow and reverse these declines.<br><br>This project aims to further the understanding of the habitat use by dormice a radiotracking study into the effects of habitat modification on dispersal, survival and home range area.<br><br>Radiotracking has previously been used to provide invaluable information on the behaviour and movements of dormice, providing details on foraging | |

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| | behaviours, habitat use, home range size, activity patterns and dispersal. Radiotracking can also provide information on the response of animals to a specific disturbance event, We aim to look at the immediate response of the dormouse population to habitat modification through felling and the alteration of habitat use and ranging behaviour. |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | This study will contribute to the body of scientific work on foraging and space use of small mammal populations. This will increase the level of understanding of mammal population demographics and the predictive power of assessing the response of populations to habitat configuration and change.<br><br>The study will also have direct policy relevance as it will contribute to best practice guidance for all forestry practitioners in the UK on how best to comply to European Protected Species legislation. This will be able to better inform both conservation management of woodland, and how best to minimise any adverse effects to dormouse populations through timber production. Thus this research will have direct conservation implications to aid declining dormouse populations in the UK.<br><br>The results will also be used in conjunction with analyses of national population trends of hazel dormice using long-term nationwide monitoring data. This will allow a comparison of long and short-term effects of woodland management. This will allow the mitigation of any detrimental impacts on individuals, while optimising the potentially benefits of habitat maintenance for population persistence. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Up to 80 hazel dormice (*Muscardinus avellanarius*) between Summer 2015 and autumn 2016. |
| 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 | Possible abrasions from a radiocollar (<5% unlikely), possible failure to retrieve radiocollars (<5% unlikely). Animals will be released back onto capture sites. |

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| happen to the animals at the end? | |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The project is focussed around a study of mammals in the wild. Therefore it is not possible to conduct this work without the use of animals, and cannot be predicted through analysis of existing information or simulation. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | In order to better characterise the effect of habitat modification in the radiotracking study control sites are being used where no management is occurring. This will reduce the number of dormice that would be needed to account for individual variations in movement. |
| **3. Refinement**<br><br>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 study aims to gather species-specific information in order to inform dormouse conservation. Aside from this fact, the dormouse represents a good model to examine the effect of habitat change as they occupy small, defined home ranges, and use a variety of habitat levels.<br><br>Radiotracking has been used to good effect with many species of small mammal including dormice, in order to gain valuable information on home range, habitat use and dispersal. Capture-mark-recapture has been used to determine information on movements and home-range use, but will not be able to give any reliable information in the short time frame. Radiocollars will never be over 10% of the weight of the animal. Every effort will be made to recapture the dormice in order to remove the collar. |

| **Project 19** | **Avian malaria in migratory and resident birds** | |
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| Key Words (max. 5 words) | Migratory bird, resident bird, blood parasite, avian malaria | |
| Expected duration of the project (yrs) | 5 | |
| Purpose of the project as in ASPA section 5C(3)<br><br>(Mark all boxes that apply) | X | Basic research |
| | X | Translational and applied research |
| | | Regulatory use and routine production |
| | X | Protection of the natural environment in the interests of the health or welfare of humans or animals |
| | X | Preservation of species |
| | | Higher education or training |
| | | Forensic enquiries |
| | | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 1) | The distribution of living organisms varies geographically and over time. This project examines whether this applies to the prevalence of blood parasites in a resident bird (Blue tit) and a migrant bird (Pied flycatcher) at a UK study site and at other breeding sites across their European breeding range. The UK represents the north western extremity of these species breeding range and so provides a contrast in climatic conditions from other study sites across Europe where complementary data is also being collected. Scientific understanding of parasite distributions and therefore disease transmission is frequently incomplete, even for well-studied groups. The data collected here is part of a project developing fine-scale maps of distribution and transmission of avian blood parasites.<br><br>We are able to simultaneously monitor infection in both Blue tits and Pied flycatchers. Blue tits remain in natal areas year round. The Pied flycatcher winters in sub-Saharan western Africa between November and late February. They then migrate across Iberia, with UK birds arriving to breed in early April. Pied flycatchers depart from breeding | |

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| | grounds after breeding in late July/early August.<br><br>Bird infection of avian malaria occurs by transmission of sporozoites in the saliva of a female mosquito bite. The sporozoites take 36-48 hours to develop into the merozoites which infect red blood cells, then reproduce asexually until cell rupture and release of merozoites into the blood stream, which causes the disease. Blood parasites first have a prepatent period (when the parasite develops in internal organs), then a primary parasitemia period when the parasite develops further, then a latent stage of infection (parasites are absent in the blood but persist in internal organs) and in some cases a secondary parasitemia due to a relapse. Costly life-stage for birds, such as breeding or low food availability may exacerbate symptoms and cause relapse. |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 2) | We will collect blood samples from adult and nestling Blue tits and Pied flycatchers to reveal the presence of blood parasites. Bloodsucking insects captured in nest boxes will be counted and identified to determine the composition of blood-sucking community and identity of potential parasite vectors. We can be 100% sure that any infection detected in nestlings result from local transmission. However, for adults it is possible that strains obtained during migration or in wintering areas will relapse during the breeding period. It is possible that these can then be transmitted in our study site, assuming appropriate vectors are present although in the absence of vectors they will not be transmitted. It is possible to differentiate between transmission on breeding grounds, on migration or at wintering grounds by contrasting parasites between the resident Blue tit and migratory Pied flycatcher, or by transmission on breeding grounds, on migration or at wintering grounds by contrasting parasites between the resident Blue tit and migratory Pied flycatcher, or by contrasting nestlings with adults of either species.<br><br>These data will be used to construct a series of models that predict the distribution of diverse infected hosts, parasites and vectors. We will then compare these models to investigate differences between years and between early and late nesting birds. From this we will create a map summarising the risk of infection. For the migratory Pied |

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| | flycatcher we will also collect a winter grown feather to use for stable isotope analysis that looks at ratios of carbon-13, nitrogen-15 and deuterium elements in the feather, which enables us to identify approximately what wintering location and diet in Africa the bird used. This will enable us to know whether the bird uses the same location each year, so we can investigate how repeatability of winter location and diet influences blood parasite prevalence in the breeding season. |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | This will be the first work to map the exact location of transmission and the spatial variation of Haemosporidian parasite distribution patterns at a high spatial resolution.<br><br>Given the severe declines seen in many migratory species, including the Pied flycatcher, and increased prevalence of blood borne parasites in birds, generating a better understanding of blood parasites species prevalence across their breeding range and transmission routes, can influence inform conservation efforts. Understanding the influence of environmental factors such as weather and habitat on blood parasite prevalence will greatly help our understanding of constraints and both individual and population level effects.<br><br>The novelty and breadth of this research will result in the findings being published in both scientific and popular publications. Results will also inform research and monitoring conducted by conservation organizations investigating population change of our focal bird species in the context of changing environmental conditions experienced by birds in spring. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Adult Blue tits and Pied flycatchers, and their nestlings, are captured during the breeding season over five springs (May-June) 2015 – 2020. Approximately 900 birds will be sampled during each 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? | Adverse effects, if any, are likely to be very mild. Blood flow will be halted using pressure and cotton wool. In the unlikely event of this failing to stop blood flow, a small amount of super glue or similar will be applied. Following sampling, all individuals will be released into the wild at the capture site. |

| **Application of the 3Rs** | |
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| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | This work could not be done using computer simulations or modelling since it requires information from animals living in the wild. For example determining vector species and seasonal timing of infection cannot be known or predicted, and so cannot be simulated. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | All sampled individuals are fitted with a uniquely numbered metal leg band so we know if the bird has been sampled previously. |
| **3. Refinement**<br><br>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. | Blue tits and pied flycatchers are well studied model systems for large-scale experiments including studies of their parasites. They make an ideal system because they breed at high enough densities to enable sufficient sample sizes to be obtained and are very resilient to interference. As such an ideal model system a network of similar well studied populations exists throughout Europe, which enables collaborative work such as this to take place.<br><br>Adult birds will be captured by licensed bird handlers and placed in a holding bag until sampling. Adult birds will be weighed, measured, aged and sexed at this point. Young are removed from the nest together to maintain the temperature of the brood and prevent chilling and are weighed and measured immediately and efficiently by skilled and practiced handlers. A single blood and (for adult birds only) feather sample will be collected per adult individual per year at the capture location. Undertaking all sampling procedures at the capture location minimises transport, handling and the duration of disturbance to the nest. The total time from capture until release of adults within the territory or replacement of chicks in the nest box is no more than ten minutes. This minimises stress to the birds, and also allows them to be released more rapidly into a familiar environment and return to normal behaviours such as the provisioning of chicks. By processing the birds within the bounds of their territory they do not need to be rehomed and due to the short duration of disturbance, i.e. no longer than the standard handling time when ringing birds recommended by the British Trust for Ornithology, no rehabilitation programme is |

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| | necessary. The pain and distress caused by our work is minimal, and highly unlikely to result in lasting harm or pain to the animal. |