Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 8

Projects with a primary purpose of: Translational and applied research – Diagnosis of Diseases

Project Title and Keywords

1. **Detection of Bacterial Toxins**

2. **Super-high Affinity Sheep Antibody Creation**
    - Sheep, Monoclonal, Antibody

<table>
<tr><td>PROJECT 1</td><td>Detection of Bacterial Toxins</td></tr>
<tr><td>Key Words (max. 5 words)</td><td></td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>The project is 5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</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[2]</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>The project enables us to fulfil the primary role for the rapid and effective investigation, diagnosis, surveillance and control of outbreaks and incidents of botulism and tetanus.</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>Results of the tests for botulism and tetanus are returned to clinical colleagues involved with the management of diseased patients and allow confirmation of the clinical diagnosis and evidence for their most appropriate clinical management and treatment. Results of tests for botulism are also used for the identification of sources of infection, and is of especial importance to those involved with control of the food chain i.e. Environmental Health Officers and staff of the Food Standards Agency. These results provide vital informed and evidence based information for the identification of toxic food and allows its removal from the food chain to prevent further cases of disease. This has far reaching health benefits for both humans and animals.</td></tr>
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	used for the identification of sources of infection, and is of especial importance to those involved with control of the food chain i.e. Environmental Health Officers and staff of the Food Standards Agency. These results provide vital informed and evidence based information for the identification of toxic food and allows its removal from the food chain to prevent further cases of disease. This has far reaching health benefits for both humans and animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Inbred Balb/c mice will be used and approximately 60-100 mice could be used over a 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?	Mice are injected with serum, faecal and food extract. If the test is positive, then the animal will be euthanized as soon as a positive result is recognised. Mice, if positive will experience these adverse effects (Botulinum) - Pilo –erection, wasp like waist, laboured breathing and paralysis. In the case of tetanus the mice will experience progressive paralysis, curvature of the tail (towards the side of the injection), limping and stiffness of limbs, total paralysis and spastic paralysis of limbs. The level of severity for this test/project is overall severe. Out of 500 test carried out over the last project 75 mice were found to be positive which is approximately 16- 20%. Of course this is taking into account that if the test is positive the animal will suffer severe pain and distress. If the test is negative then the severity would be mild/ moderate retrospectively. The retrospective assessment would reflect this. All animals are euthanized humanely at the end of the test. Animals are closely monitored throughout the duration of the test and a member of staff is on call 24 hours a day.
Application of the 3Rs	
1. Replacement State why you need to use	Most of the work detected botulism and tetanus is carried out in the laboratory, however there are

animals and why you cannot use non-animal alternatives	occasions when the use of animals is necessary. Sometimes we cannot get a clear or definitive result using PCR or lab based equipment and therefore the reliable method of ascertaining if the sample is positive or negative, is to use animal testing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Relatively large amounts of toxin will be present in culture supernatants i.e., floating above or on the surface of the organism growing *in vitro/in the laboratory,* and we have made considerable progress in the use of the polymerase chain reaction (PCR) as an alternative to animal tests. However, there will still be a need occasionally for animal *in vivo* tests applied to cultures growing *in vitro/ in the laboratory,* since the PCR may not detect organisms with unusual toxin/ disease types or toxin/disease variants. Out of 500 test carried out over the last project 75 mice were found to be positive which is approximately 16- 20%.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Testing protocol is designed to minimise the number of animals used. Where possible tests will be carried out in the lab without the use or need for animal tests, However there are occasions when we cannot ascertain if the sample is negative or positive and mice have to be used in order to establish whether the sample is negative or positive and we will also be certain of the strain once we correlate the lab results to the animal result. Mice are the lowest sentient/ mammalian species we could possibly use. We use Balb/c mice for continuity in our test as these have been used historically they do not gain weight as rapidly as other strains or especially outbred mice would gain weight, which would/could adversely affect the test. Mice are given wet diet and extra bedding to make them more comfortable during tests whether the test is found to be negative or positive.

PROJECT 2	**Super-high Affinity Sheep Antibody Creation**	
Key Words (max. 5 words)	Sheep. Monoclonal, Antibody	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals[3]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To create super-high affinity sheep monoclonal antibodies for use in clinical diagnostics (ie. blood tests, urine tests, serum tests, etc.). These tests might take place in hospital laboratories or at “point-of-care”.	
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)?	Our high affinity sheep antibodies have been proven to give rise to more sensitive & accurate diagnostic tests, enabling clinicians to better diagnose, monitor & therefore treat patients. Many diagnostic tests still use polyclonal antibodies (derived from repeated immunisation & blood sampling of animals) which require constant use of animals over time. Monoclonal antibodies (derived from animal cells grown in the laboratory) only require animals to be used once during their production, Therefore, by replacing the antibody element of a diagnostic test with a monoclonal antibody, fewer animals will be used in total.	

What species and approximate numbers of animals do you expect to use over what period of time?	Sheep; expect to use approximately 200, maximum 500 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All methods used are classified as “mild” severity by Home Office classifications. A relatively low number of animals experience localised inflammation/abcessation as a result of the procedure but do not display any signs of discomfort. All animals are euthanased humanely by a vet following procedures, in line with Home Office requirements.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives do not produce antibodies of sufficiently high sensitivity/affinity. Antibodies are large proteins with complex structures; it is not currently possible to create correctly formed artificial antibodies, let alone those with high affinity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Extensive experience in immunising sheep to give rise to high-affinity antibodies and constant data monitoring ensures the number of animals used per project is sufficient but not excessive.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Large mammals have a larger diversity of white blood cells than rodents (mice are most commonly used for antibody production). This allows us to make antibodies that others cannot. Of large animals, sheep are suitable because of: • Ease of immurilsation and blood sampling procedures — usually remain placid during procedures. Whilst sheep do not like to be singled out, they remain calm so long as they are within sight of other sheep and are in no visible pain. Procedures are completed very quickly (usually within seconds), so any stress of being restrained is transient. • Ease of housing — can live a “natural” life on a farm.

	• Availability of a cell line in our laboratory that allows us to make the sheep's white blood cells (which produce the antibody) "immortal". In work done in previous projects, refinements to how we prepare immunisation sites, choice of where to immunise and the technique used have minimised inflammation. Monitoring sheep for general welfare and for any adverse reactions is regularly conducted.