Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 16

Project Titles and key words

- Reducing the environmental impact of methane emissions

  Sheep, cattle, forages, grazing, methane

- Understanding brain systems

  Addictive Drugs Reinforcement; Reward

- Hormonal and growth factor control of breast cancer

  Breast Cancer, Hormones, Novel Therapy, Clinically relevant, endocrine resistance

- Models of breast cancer heterogeneity and biomarkers

  Breast Cancer, Metastasis, Treatment, Diagnosis,

- Cellular stress and drug metabolism in disease

  Drug metabolism; oxidative stress; cancer

- Glucocorticoids and cardiovascular Disease Risk

  Steroids, inflammation, atherosclerosis, diabetes, obesity

- Reagent production and screening

- Poultry production and welfare

  Gut health, feeding, osteoporosis, eggs, reproduction

- Genetics of poultry development and welfare traits

Reducing the environmental impact of methane emissions

Sheep, cattle, forages, grazing, methane

- Summarise your project (1-2 sentences)

This project will quantify methane emissions from different age classes and breeds/genotypes of cattle and sheep when managed a range of farm systems, and identify ways in which these emissions can be reduced.

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

Methane is a significant contributor to greenhouse gases and hence to global warming. Agriculture is the source of about 38% of total UK emissions of methane, and of this about 85% came from livestock enteric sources (mostly ruminants). The current UK National GHG Inventory largely estimates emissions from agriculture using the most simplified approach to accounting (Tier 1 of the United Nations Framework Convention on Climate Change (UNFCCC) reporting system). Using this method GHG emission data are compiled using default emission factors for the various livestock categories and their manures. This approach uses livestock population numbers multiplied by a standard factor; does not differentiate between standard practices, new or innovative processes; and takes no account of any mitigation practice designed to reduce GHG emissions. In order to reduce this uncertainty, and to track the effects of efforts to mitigate methane outputs, it is necessary to move to the more sophisticated Tier 2/Tier 3 accounting methods. These use disaggregated emission factors combined with farm business activity data, and incorporate production system-specific data. However there are substantial gaps in the UK applicable data currently available. The experimental work covered by this Project Licence has been designed to target the main areas of livestock production in which little previous work has been done in order to quantify enteric methane emissions from sheep and cattle fed standard diets, and identify strategies to reduce these. While the research will focus on methane emissions, data on the wider impacts of livestock farming will also be collected in order to provide a sound evidence base for the development of future rural policies.

- Outline the general project plan.

The required data will be generated through feeding trials or zero-grazing studies with housed animals, or though grazing experiments with animals at pasture. Experimental design is of utmost importance in ensuring that the results generated are statistically strong. Fit and healthy animals will be selected based on parameters such as gender, age, physiological state (barren, pregnant or lactating), body weight and body condition score. Animals may be kept in pens or in a field. Experimental dietary treatments will then be imposed in an appropriate sequence to allow correct statistical analysis of the results. Following the appropriate adaptation period, data and samples will be collected in order to quantify feed intake, liveweight change, gaseous emissions, metabolic status and feed digestibility. At the end of an experiment comprising a set of regulated procedures, animals will be inspected by a vet or other suitably qualified individual and, if certified fit (i.e. not suffering pain, distress or lasting harm as a result of the procedures) will be released back into the main herd/flock.

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

In order to determine voluntary intake, methane emissions and diet composition, inert markers (e.g. *n*-alkanes, SF_6) may be administered by dosing gun and faecal grab samples taken. Blood samples may be taken by percutaneous venepuncture to monitor the effect of diet consumed on metabolic status. Halter/collar-mounted recording/sampling devices may be fitted to allow grazing behaviour to be monitored and/or samples of gases released via the nose to be collected. Some procedures may cause minor discomfort at the time, but there should be no lasting harm.

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

The results from this research will be of direct and immediate benefit to UK and EU policymakers and will directly contribute to the UK and DA Governments' efforts to meet UK and international targets for reductions in GHG. The results will also be of benefit to the UK farming industry as they will establish accurate values for current emissions, and allow the industry's efforts to mitigate GHG emissions to be recognised. Reductions in enteric GHG emissions are generally linked to improvements in production efficiency and thus the general public will benefit from food being produced in a more cost-effective way.

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

Around 400 sheep and 200 cattle will be used. They are the objects of study and are not being used as a model for any other species. The preparation of all experimental protocols involves consultation with statisticians, who provides advice on issues such as the minimum number of animals required to make the results from any particular experiment statistically and biologically significant. The results of previous work (ours, or from the literature) are used to carry out power calculations for the optimum number of animals required for use in an experiment of any design.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

In grazing and growth studies it is not feasible to gain the results satisfactorily from any method not entailing the use of protected animals. These studies integrate behavioural, ingestive and metabolic parameters and no suitable procedures (either *in vitro* modelling or computer prediction models) exist to attain the required results.

- Explain why the protocols and the way they are carried out should involve the least suffering.

In order for the data to be representative the conditions in which the animal will be studied are designed to be as close as possible to those used in commercial livestock production systems. Any suffering would alter the behaviour of the animals and render the results unrepresentative.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Understanding brain systems</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Addictive Drugs Reinforcement; Reward</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>(1): Understand how addictive drugs such as nicotine and amphetamine act in brain to influence behaviour.

(2): Identify areas of the brain that could be targets for drugs that can be used to help withdrawal from nicotine and other drugs.

(3): Identify how low and high level parts of brain work together when learning is happening.</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 benefit will come from understanding how the interaction of "lower" and "higher" systems in brain works in relation to learning. This work is new in two ways – first, the integration of structures at relatively low levels of brain with those higher up, rather than the typical focus only on the "higher" systems"; and second, the identification of a particular part of brain – the hippocampus, which is closely associated with memory formation and storage – as a target for the effects of nicotine (and potentially other drugs of abuse).

The other main benefit comes from examination of nicotine. Nicotine is a drug of abuse and the consequences of its use adds very substantially to health and social services around the world. Using intracranial self-administration – rats delivering minute quantities of drug directly into their own brains – enables us to determine if there are particular "hot spots" for nicotine's effects. As we identify areas that are strongly associated with the effects of nicotine we can attempt to target other drugs to these sites that might help people stop smoking.</td></tr>
</table>

	More generally understanding how these particular brain systems work may help with other diseases such as schizophrenia, depression and Parkinsonism.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult rats will be used in this project we expect that approximately 1725 will be used over 5 years in these studies.
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 main adverse effect on the rats comes from the surgery. There is always risk that there will be pain and discomfort, for which we give the animal pain relief. The banding for most procedures is moderate – the risks to the rats can be reduced through good technique and post operative care, such as twice daily checks and giving them soft food. One procedure is banded as severe, but will be used only for very specific purposes, when needed. These animals are monitored more often, we have a daily check list (a score sheet) where we record how the animal is progressing. The animals will experience weight loss, some loss of balance, some pain and hunching, but by seven days after the operation we expect them to be healthy again. Typically at the end of the study rats are humanely killed by drug overdose to preserve tissue in a natural state. This is necessary because we will need to examine brain tissue so that relationships between any brain changes and the behavioural effects the rats showed can be understood.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rats are the subjects of this research in which drugs are targeted directly into the brain by the rats themselves – a technique termed intracranial self-administration (ICSA). At present there are no alternatives to using rats as subjects when using ICSA. We believe that the likely benefit in terms of addition to knowledge and potential practical benefit make the use of rats in these studies justified. Given the similarity across species of the brain areas we are interested in, the results will translate well to other animals and humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We do statistics to find out the right number of animals to use for each study to give us the answers we are looking for. To minimize the numbers, we carry out pilot studies to determine target brain co-ordinates: these are extremely valuable in reducing the total number of rats required. Techniques to be used in this project are

	routinely used in this laboratory and are continually refined to keep animal use to a minimal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats are the subjects in which drugs are targeted directly into the brain by the rats themselves. Rat brain develops and is organized in a strikingly similar way to human (and other vertebrates). The essential connectivity and functionality of structures below the cerebral cortex is strikingly similar – and even in the cortex there are clear points of comparison such that cortical – subcortical connectivity is essentially the same. Rats are well suited to behavioural experiments and to the research we will do here. The avoidance of animal suffering is central to this work – experiments in which behaviour is being measured do not produce reliable data if animals are at all unwell. Surgery will cause some post-operative pain or discomfort. We use pre and post-operative pain relief to reduce discomfort after surgery and rats are monitored at least daily; they may also be offered soft food or sugar in the water supply after surgery. staff are trained to be able to see when the animal is in pain or discomfort and will then take action. We have improved the way the pumps are anchored on the rats, significantly improving the pumps' endurance and reliability (which helps keeps animal numbers to a minimum). A combination of experience, literature searches, taking the vets advice and advances in equipment help us continually to refine techniques.

Hormonal and growth factor control of breast cancer
Breast Cancer, Hormones, Novel Therapy, Clinically relevant, endocrine resistance

- Summarise your project (1-2 sentences)

  The majority of breast cancers (BC) use oestrogen (E) for their growth. Patients diagnosed with E-responsive primary BC are treated with anti-oestrogens, which block the E-signalling pathway and therefore tumour progression. Although effective, around 40% of patients metastasise. Our aim is to identify the genes and proteins that change in tumours that become resistant in order to identify novel drug targets and new clinical strategies

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

  We have carried out extensive research in our laboratory using cell lines grown on plastic in an attempt to model resistance to anti-estrogens. These studies have shown that certain molecules that control cell growth are increased when the tumour cells become resistant to therapy. We have used new drugs that target these and shown that by blocking these molecules we can make the tumour cell respond to anti-oestrogens again. Although these experiments provide insight into which molecules and drugs are useful they do not model the tumours "microenvironment" e.g. how the tumour makes a blood supply or how tumour cells are affected by surrounding normal cells. To do this we need to place human tumour cells into *immunocompromised* mice allowing us to model the tumour in the patient. These studies cannot be carried out immediately in the clinic, as we must first be certain that these new drugs are effective at inhibiting tumour progression. Those drugs that are successful can then be tested clinically

- Outline the general project plan.

  Our general sequence of work is as follows: In the laboratory we engineer tumour cells to become resistant to anti-oestrogen therapy. Using growth assays and molecular analysis we identify new drug targets. Once we have established this, we implant the resistant human breast tumour cells (or their parental cell line) into a small number of immunocompromised mice.

  We use female immunocompromised mice, which have a deficiency in their immune system allowing them to accept and grow human malignant tissue.

  The ovaries are removed under anaesthetic and breast tumour cells are then implanted under the skin on one flank or both flanks of the animal. Mice are then given E or its precursors, modelling the hormone level in a patient suffering with BC.

  Once the tumours have developed. Mice are divided into groups of equal number and given daily therapy by oral dosing for up to 28 days. The therapeutic agents' potential toxicity is monitored via changes in the weight and body condition of the mice.

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

  Cancer cells are allowed to grow under the skin of the mouse on the flank. These cells

are either given by injection or by implanting a small fragment of tumour via a small incision. Agents which support the tumour growth are given via injection under the skin. Therapy drugs are given by either an oral gavage, or an Intraperitoneal injection. All procedures are carried out by skilled technicians and using aseptic techniques. This reduces the risk of any adverse effects occurring as a result of the procedure. Chemotherapeutic agents are known to have some adverse effect on the animal. These are minimised by using doses which have been determined as being suitable to use in these animals for the duration of the studies. Any adverse effects are managed and dealt with promptly.

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

  These studies will allow us to characterise mechanisms of resistance to endocrine therapy, to identify biomarkers that can identify patients likely to relapse and allow us to develop new clinical strategies to treat these patients

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

  The number of animals necessary for a typical combination study of 6 treatment groups is 70. A minimum of 10 animals per group are necessary since in the Wilcoxon matched paired test, the most rugged statistical test for comparison within groups, requires a minimum of 6 animals for a change to be deemed statistically significant. Given that a small number of animals may not develop tumours, 10 animals per group are necessary
  Athymic nude mice are used as the model for these studies. These animals have a limited immune response which allows tumours to grow without the animals immune system affecting the growth and therefore the scientific data produced.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

  We have carried out extensive research in our laboratory using cell lines grown on plastic in an attempt to model resistance to anti-estrogens. We have used new drugs that target these and shown that by blocking these molecules we can make the tumour cell respond to anti-oestrogens again. Although these experiments provide insight into which molecules and drugs are useful they do not model the tumours "microenvironment" e.g. how the tumour makes a blood supply or how tumour cells are affected by surrounding normal cells. To do this we need to place human tumour cells into *immunocompromised* mice allowing us model the tumour in the patient. These studies cannot be carried out immediately in the clinic, as we must first be certain that these new drugs are effective at inhibiting tumour progression. Those drugs that are successful can then be tested clinically.

- Explain why the protocols and the way they are carried out should involve the least suffering.

  Each experiment is designed to use the minimum number of animals over the shortest period of time to enable us to obtain clinically relevant data. As soon as this data is obtained the animals are humanely killed. All procedures are carried out by skilled technicians under controlled aseptic laboratory conditions. Animals are provided with analgesia during and after surgical procedures. Common injection procedures are also carried out under aseptic conditions.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Models of breast cancer heterogeneity and biomarkers</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Breast Cancer, Metastasis, Treatment, Diagnosis,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
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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>Breast cancer is the most commonly diagnosed cancer in the UK; 12,000 women die each year due to this disease. Breast cancers are broadly classified into distinct types by expression of combinations of the oestrogen receptor (ER), growth factor receptors and other biomarkers such as basal cytokeratins. Recent data have suggested that there may in fact be as many as 10 different types of breast cancer that may have different biological “drivers”. As is the case with other cancers, breast cancer is not curable if cancer cells spread to other parts of the body and multiply to form new tumours at these sites (development of metastases). A type of breast cancer with high frequency of metastatic development is triple negative breast cancer (TNBC). TNBC is defined as negative for ER, progesterone receptor (PR) and HER2. TNBC, unlike ER-positive and HER2-positive tumours, currently has no specific targeted therapy. It is thought that there are 6 types of TNBC. We aim to identify the biological mechanisms that cause and drive these different TNBCs (around 15-20% of all diagnosed breast cancers) leading to the identification of novel therapy targets, prognostic factors and biomarkers for treatment and prognosis of TNBC. We are studying TNBC using state of the art laboratory techniques (molecular, genetic, pathological) in combination with the proposed in vivo experiments in this project.

The aims of this project licence application are to establish and expand clinically relevant models of</td></tr>
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[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

	human breast cancer. We aim to establish these models in the context of breast cancer stem cells (CSC) and triple-negative breast cancer (TNBC). These models will allow us to discover new treatments for both primary breast cancer and metastasis. TNBC is a very aggressive disease, leading to metastatic spread very quickly after diagnosis of a primary breast tumour. This maybe is due to a cancer stem cell population driving tumour growth and metastatic spread that is not targeted by conventional means. We are aim to determine the cancer stem cell component of these invasive breast cancers and develop new therapies to targets these populations specifically.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In summary our project aims to identify new targets for therapeutic intervention in a very aggressive form of breast tumours that will potentially translate into new treatments for human breast cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of the 5-year project we aim to use up to 3000 female immunodeficient mice. We will also use up to 1000 female mice of transgenic models that produce spontaneous mammary tumours. This number is subject to variation following results from our pilot studies. All animals will be humanely killed by Schedule 1 methods, unless otherwise specified, at the end of each protocol.
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?	Firstly we will need to establish xenograft models of breast cancer that model all the different types of breast cancer, including those of breast cancer stem cells (CSC). Xenografting is establishing human tumours derived from patient material (obtained through informed consent) in mice that do not have a proper functioning immune system (immunodeficient). The use of these specific species is justified by its ability to accept transplantation of foreign tissue due to its depressed immune system. Xenografting will be done by “humanizing” the mammary glands of female mice and by injecting the humanized glands with human breast tumour samples. Every invasive procedure will be performed under general anaesthesia with analgesia given afterwards to ensure minimal distress is caused to the animal. The growth of tumours will be closely monitored over time and it will be made sure the animal only incurs the minimum possible discomfort and pain.

	Secondly we aim to identify what therapeutic agents are effective against our tumourigenic targets identified from our laboratory studies into the subtypes of breast cancer, particularly those that promote the growth of TNBC. As part of this project we will be extensively testing our targets in the laboratory in tissue culture models and will only test those targets that affect a “hall mark” of cancer in the laboratory experiments before moving into our xenograft models as a prelude to taking them on into human drug and prognostic biomarker development studies in humans. This will ensure the minimum number of animals are used in these studies. In order to identify these therapeutic agents we will treat with established and novel agents human tumours grown in mice (xenograft) and also transgenic mouse models of spontaneous breast cancer that mimic human TNBC. We will correlate the tumour’s response (both human and transgenic) to such treatment with the presence and characteristics of our TNBC and CSC markers. At present it is necessary to perform *in vivo* studies as an intermediate step in the translation of laboratory findings to the human clinical setting. Tissue culture models cannot replicate, as yet, the complex interactions between bulk tumour cells, CSC, tumour stromal cells, including immune cells, and the blood vessel network. In particular when examining the response of tumours to therapy, the utilization of an *in vivo* model provides the advantage of generating many tumours of the same or different type that may be analyzed simultaneously to examine molecular and genetic profiles correlating with clinical response. Thirdly we aim to identify targets and new therapeutic agents that will block the spread of cancer cells to other parts of the body and formation new tumours at these sites (development of metastases). This is overwhelming cause of death in most breast cancer patients. TNBC is a very aggressive disease and metastatic spread occurs sometimes simultaneously with diagnosis of the primary breast tumour. We aim to use models of metastatic spread into the blood circulation system and another organ, bone marrow (tissue found inside bones that produces new blood cells). The development of metastasis will be closely monitored overtime using imaging techniques where possible and it will be made sure the animal only incurs the minimum possible discomfort and pain. We have developed *in vitro* models of bone marrow that we will use to screen new therapeutic

	targets before moving to *in vivo* validation experiments. This should ensure a reduction in the number of animals used in our experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present it is necessary to perform in vivo studies as an intermediate step in the translation of laboratory findings to the human clinical setting. Tissue culture models cannot replicate, as yet, the complex interactions between bulk tumour cells, cancer stem cells, tumour stromal cells, including immune cells, and the blood vessel network. In particular when examining the response of tumours to therapy, the utilization of an in vivo model provides the advantage of generating many tumours of the same or different type that may be analyzed simultaneously to examine molecular and genetic profiles correlating with clinical response.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot studies will be conducted prior to every protocol in order to assess the suitability of the scientific procedure and to reduce to the minimum the number of animals likely to suffer distress or pain. It is important to stress that in vivo models will not be used for random compound screening and all experiments will be hypothesis-led on the basis of extensive laboratory studies, established clinical practice or currently ongoing clinical trials. The number of animals will be kept to the minimum needed for a statistically valid result. We have developed in vitro models of bone marrow that we will use to screen new therapeutic targets before moving to in vivo validation experiments in models of metastasis. This should ensure a reduction in the number of animals used in our experiments.
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.	Initially it is our intent to use non-obese diabetic/SCID (NOD/SCID) and NSG (NOD/SCID/gamma) female mice in our studies. The use of these specific species is justified by its ability to accept transplantation of foreign tissue due to its depressed immune system. However, other immunodeficient strains may be used if found to be more appropriate. Every invasive procedure will be performed under general anaesthesia with analgesia given afterwards to ensure minimal distress is caused to the animal. The growth of tumours and development of metastasis will be closely monitored overtime, using imaging methods where possible, and it will be made sure the animal only

	incurs the minimum possible discomfort and pain.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Cellular stress and drug metabolism in disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Drug metabolism; oxidative stress; cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Our main aims are to understand the systems that protect us against toxic agents, both from our environment – the air we breathe, the food we eat, the medicines we use – and from within our own cells as a result of natural processes. It has been argued that the most important areas for expression of xenobiotic (drug) metabolising enzymes are those which are in contact with the environment, ie the skin, the gi tract (from top to bottom) and the airways. A number of molecules of interest are highly expressed in the pulmonary epithelium to protect against airborne chemicals, consumed deliberately or as part of our environment. The current health worries from the smogs in Asian cities are testament to the importance of airborne chemicals. By appreciating how these systems work, we want to take this knowledge and use it to enhance how we design and administer anti-cancer drugs, with a view not only to improving efficacy and a personalized response to treatment but also circumventing issues like drug resistance.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The transgenic mouse models used in this Project will be invaluable in acquiring more knowledge concerning the cellular mechanism(s) involved in protection against environmental chemicals, and how these systems relate to disease susceptibility and resistance to drug treatments. This will lead to a greater understanding of individuality in response to drug treatment; one of the major benefits we expect to achieve through this work is the optimisation of existing anti-cancer drug therapies, and the development of novel treatment strategies based on emerging drugs.</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

What species and approximate numbers of animals do you expect to use over what period of time?	Our work will be exclusively in mice, and we expect to use approximately 25000 animals over the 5 year period of the licence. The vast majority of these mice will be used in breeding programmes to generate appropriate genotypes and for the harvest for tissues, and only a small proportion will be subject to treatment.
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?	Apart from breeding and maintenance, some of the mice will be treated with agents which may have mild to moderate short-term effects. Some experiments will involve growing human tumours in order to study the effects of drug treatments; however, such tumours will not be allowed to grow to a point where they significantly affect the well-being of the animals. These, and other mice, may have multiple blood samples taken over a period of time, for example to allow measurement of drug levels in the blood; this will be done in a manner that minimizes stress to the mice, and in fact will result in the acquisition of higher quality data from a significantly smaller number of animals (see 'Reduction' below). At the end of experiments or at the end of their useful breeding life, mice are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible, we make extensive use of alternative experimental resources, including computer simulations and work with cell culture models. However, in order to adequately assess drug efficacy in relation to humans it is important to remember that, in addition to drug metabolism, the disposition of drugs, i.e. how they are distributed throughout the body, absorbed from the gut, excreted in bile/urine/faeces, is central to understanding how drugs work and how therapeutic action is related to side-effects. Thus, it is essential to use a multi-compartment experimental model such as the mouse.
2. Reduction Explain how you will assure the use of minimum numbers of animals	At all times, experimental design will be such as to, maximise the information obtained from the minimum number of animals. For example, in our pharmacokinetic work, we have pioneered serial bleeding techniques, which together with simultaneous multi-drug dosing and the use of analytical instruments with increased sensitivity, has reduced our animal use in many of our experimental protocols by a factor of up to 50-fold, whilst generating data of a significantly increased quality. Further, we have also designed and validated a number of new mouse lines in which reporter expression can be monitored and measured non-invasively, again significantly reducing animal usage.

	We continue to seek ways in which we can reduce animal numbers and refine our protocols without compromising the results obtained. Our experiments are always designed to maximize data output from the lowest possible number of animals without sacrificing scientific ;
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice, whilst displaying a number of physiological differences to humans, represent a good compromise for experimental purposes. They have a relatively short gestation period, allowing the rapid generation of animals. Mice are also amenable to the sort of genetic manipulations that allow the creation of unique experimental models, including humanization for key enzyme systems, thus generating data with greater relevance to Man. the endpoints are chosen in a similar manner.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>GLUCOCORTICOIDS AND CARDIOVASCULAR DISEASE RISK</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Steroids, inflammation, atherosclerosis, diabetes, obesity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</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[6]</td><td>Yes</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Glucocorticoids (steroid hormones) are the mainstay of anti-inflammatory treatments, being widely used to control inflammatory diseases such as asthma, rheumatoid arthritis, and inflammatory bowel disease. However, with long-term use, glucocorticoids cause serious side-effects, increasing risk of cardiovascular disease (eg. atherosclerosis) and conditions closely associated with heart disease (obesity, type 2 diabetes, hypertension and liver disease that is not caused by alcohol) - in themselves, paradoxically, inflammatory conditions. How glucocorticoids decrease one type of inflammation whilst increasing another remains unknown but there is considerable pharmaceutical interest in identifying novel drugs which separate the beneficial anti-inflammatory effects of glucocorticoids from the harmful cardiovascular effects.

The body also produces its own glucocorticoid hormones to control inflammation and help the body cope with stress. The delicate balance that normally controls the activity of these natural glucocorticoids goes wrong in conditions like type 2 diabetes and obesity, but how and why this happens is not understood. In addition, before birth, too much glucocorticoid (eg through maternal stress) predisposes that individual to inflammatory and cardiovascular disease in adult life.

There is a scientific and clinical need to understand how the body’s own, as well as prescribed glucocorticoids, shape immune and metabolic responses, and in unborn babies,</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

	increase the risk of developing cardiovascular disease in later life. Here, we plan to research this in rodents (rats and mice).
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 research will contribute to our understanding and knowledge of how these important steroid hormones work and are controlled. Knowledge resulting from this research may contribute to refinements in clinical use of the glucocorticoids already licensed. This research might identify novel applications of glucocorticoids or could potentially lead to development of new drugs. Our research on glucocorticoids has already led to the development of new drugs currently undergoing clinical trials in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, 1000 per year; 5000 over 5y Mice, 4000 per year; 20000 over 5y
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?	Most of the animals will be used in breeding programmes. *For experimental animals:* *The inflammatory models to be used (models of sterile peritonitis, arthritis, lung inflammation and liver fibrosis) use an irritant or some other trigger to cause inflammation but this inflammation resolves by itself once the trigger is discontinued. All are well tolerated; none cause mortality. Any animals exceeding the severity limit will be humanely killed.* *Substances will be administered at doses known to be non-toxic, based on experience and dosages reported in the literature, and at volumes in accordance with best practice*. Bodyweight and behaviour (e.g. lack of grooming, coat condition) of animals receiving substances will be routinely monitored. If weight loss exceeds 20% in a 72h period, animals will be humanely killed. *For blood sampling, to avoid hypovolaemia or anaemia, not more than 10% of the total blood volume will be withdrawn on any one occasion and no more than 15% of the total blood volume in any 28-day period*. *During surgery (removal of the adrenal glands or the gonads, or implantation of devices such as mini-pumps for controlled release of substances),*

	deaths resulting from anaesthesia or surgical complications are uncommon and will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post surgery e.g. use of heat pads. Pain will be controlled during surgery by general anaesthesia and post surgery by analgesics. Post surgical infections can occur in ~1% of animals. Risk of infection will be minimised by good surgical and aseptic techniques. Surgical sites will be monitored for signs of inflammation and infection. Antibiotic cover will be given under the advice of the NVS if required. *Best practice guidelines for surgery/post-surgical care, anaesthesia and analgesia will be followed at all times.* *Immune cell depletion may increase risk of infection.* Animals undergoing prolonged immune cell depletion (>1 week) will be housed in IVC cages and may be treated prophylactically with antibiotics, under direction of a veterinary surgeon. *Bone marrow depletion and Reconstitution may involve irradiation.* Potential adverse effects of irradiation include diarrhoea, tooth damage and weight loss and bacterial infections. Mice are monitored closely and are given antibiotics before and/or after irradiation (under veterinary advice) to prevent infection. Following irradiation, food is given as mash to circumvent tooth problems and minimise weight loss. *Very few mice die of the immediate effects of irradiation (within a few days). However, reconstitution failure can result in mice becoming ill around 2 weeks after injection of donor cells when mice become sick. Mice will be monitored closely following donor cell injection for signs of illness* (eg ruffled/matted fur, hunched appearance, lack of movement in the cage and failure to move when disturbed) and those showing symptoms without improvement will be humanely killed on the advice of the NVS. In the case of an animal of particular scientific interest, we shall agree a way forward in consulation with the NVS. *Insulin tolerance tests (ITT). Hypoglycaemic shock (fitting, seizure) is a possible adverse effect. Animals will be monitored throughout the ITT and humanely killed if hypoglycaemic shock occurs.* At the end of experiments, animals will be humanely killed and tissues collected for analysis.
Application of the 3Rs	
1. Replacement	Cardiovascular disease in humans progresses

State why you need to use animals and why you cannot use non-animal alternatives	slowly (typically over 20-40 years) and is dependent on many different body systems. Nutrition, hormones and immune cells are all important. Animal models of cardiovascular disease and inflammation recapitulate key aspects of the disease in humans. The use of genetically modified animals as well as interventions that are not possible in humans allows us to dissect the different contributions of hormones, nutrition and immune cells to cardiovascular disease progression. This research can provide vital proof of concept data to enable translation of key concepts to humans. Our investigations in live experimental animals are supported by extensive analyses of tissues taken once the experiment is complete and are complemented by invetigation of isolated cell systems. Where possible, we include in our analyses human tissue samples or cell cultures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used in our investigations is based on power calculations to determine optimum group size and statistical power. Where possible, a multi-factorial design is used to increase power and reduce the overall number of animals required. The use of inbred mice reduces experimental variability and thus overall numbers required. Imaging techniques (similar to those used in humans) in live animals allow sequential non-invasive measurements, providing repeated measures within a single animal, increasing statistical power and reducing the number of animals required for experiments. The effects of treatments are based on comparison with appropriate control and/or sham treated groups. Study design is based on current best practice and, where necessary, following discussion with statisticians and/or bioinformaticians
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The ease of genetic manipulation in mice makes them a powerful tool in which to investigate mechanisms of cardiovascular disease, particularly those that impact the immune system. Genetically modified mice, especially those fed a high cholesterol “western” diet, provide arguably the most relevant and versatile models of cardiovascular disease available. In all our experiments we are mindful of the need for refinement to reduce suffering, and appropriate modifications to protocols are incorporated where possible. In carrying out an experiment, when we identify ways to reduce animal suffering without

	compromising the scientific integrity of the experiment, we will always seek to incorporate these refinements. We have already done this in several of our protocols, for example, reducing time of anaesthesia required, reducing the degree of inflammation an individual animal experiences or feeding animals mashed diet to alleviate weight loss due to tooth problems caused by the procedure.

Reagent production and screening

- Summarise your project (1-2 sentences)

We aim to produce effective veterinary immunological research tools (reagents) which will aid the further study of avian and mammalian animal models and systems.

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

Currently there is very limited availability of research tools (reagents) that enable researchers to study veterinary species. This lack of availability hampers research into diseases of ruminants, pigs and chickens. By understanding the normal physiology of these species and their responses to infection or disease research will contribute significantly to global food security and increased animal, and human, health.

This project aims to generate additional important reagents or tools to facilitate the study of immune responses in veterinary species. By providing a service to produce further reagents this project will progress veterinary research. These reagents could provide the means to develop screening tools or diagnostic tests for animal diseases and could assist with vaccine development and design.

- Outline the general project plan.

Mice, rats or rabbits will be immunised with specific proteins (antigens) and regular blood samples taken to establish their immune response to the antigens. After a suitable response has been seen the animal will be culled and blood and spleen taken in order to generate antibodies: a specific type of reagent that can then be used to define immune responses in the species of interest (including cattle, sheep, pigs or chickens). Blood will be harvested from various species in order to screen the antibodies generated for specificity and cross reactivity (whether they work on more than one species of animal).

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

No adverse effects are expected in any animals used within this study. We have extensive experience of similar studies performed over a number of years. During blood sampling only limited amounts of blood will be taken to avoid putting the animal at risk of anaemia. In some cases we may use specific compounds (adjuvants) to boost the response to the antigen being injected. These are all well tested and have not been shown to produce any ill effects in treated animals. All animals will be closely monitored throughout this study for any deviation from optimal condition.

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

These reagents will be made available to universities, research establishments and industry in order to support the study of scientific research such as:

1. Diagnosis of animal disease
2. The understanding of animal disease and physiology

Ultimately the studies which will be facilitated by the generation of new reagents will lead

to improvements in animal health and welfare and will contribute to global food security which is increasingly important with the predicted growth in the world's human population.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Poultry production and welfare</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Gut health, feeding, osteoporosis, eggs, reproduction</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project aims to improve reproduction, product quality, welfare and sustainability in meat and egg type poultry. The combination of egg and meat production make the chicken the foremost source of animal protein in the world and in the case of eggs an almost perfect balance of protein for human nutrition. The widespread availability of this high quality protein has been the result of both genetics and systems of management. Some of this has progress has had unintended consequences on both welfare of the bird or quality of the product.

In this project we are addressing issues which influence both quality and welfare.
We seek to understand the control of food intake in chickens. This is because food restriction is currently required to maintain reproduction in meat type birds.
In egg laying birds osteoporosis during the egg laying period can lead to bone breakage particularly in extensive systems.
Poor egg quality is a major reason for the culling of egg laying hens and increases the risk of microbial contamination as well as inter-generational pathogen spread.
The removal of therapeutic antibiotics has produced a major challenge to the gut health of rapidly growing poultry.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>By understanding the mechanism of feed intake we can devise strategies which can control growth whilst maintaining reproductive output and the</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

advanced or humans or animals could benefit from the project)?	level of satiety of the birds. By understanding the genetics and physiology of what leads to bone weakness we can help select hens or derive nutritional strategies which will reduce the chances of bone breakage. By devising new measurement strategies and understanding egg formation we can improve the selection of hens and reduce waste, increase biosecurity and protect consumers. By identifying natural antimicrobial molecules we will be able to test new strategies to improve gut health in poultry
What species and approximate numbers of animals do you expect to use over what period of time?	Chicken (~770) and Quail (~150)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	It would be expected that for around 90% of the animals only an injection or change of environment and blood sampling will performed with a mild severity. Apart from minor discomfort or stress no adverse effects are expected. For a small proportion (<10%) a surgical procedure with a moderate severity will be performed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying complex interactions of environment and multiple organ systems, e.g. bone and oviduct in the production of eggs, which cannot be studied in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers used are determined by a calculation which takes account of the size of differences we expect to observe. This ensures the numbers are appropriate.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In general we do not consider the animals as models, but for their intrinsic worth as a source of food. Most experimentation will involve mild stress or discomfort however possible we will use administration methods which minimise handling. In the case of surgery analgesics will be used

Genetics of poultry development and welfare traits

- Summarise your project (1-2 sentences)

  The overall aim of the project is to investigate the genetic basis of aspects of embryonic development, disease and commercial welfare in poultry. There are two parts to the project: the first investigates genes that cause major loss of function and disease (including blindness) and have relevance to human conditions; the second is designed to study the genetic and environmental basis of traits affecting poultry development and welfare.

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

  In the first part of the project we use unique mutations in the chicken to study the development of limbs and sight in the embryo and early life. Embryonic limb growth is studied in a natural mutant chick line as a model of human diseases associated with similar pathological mechanisms. The chicken eye is very useful as it is more like the human eye and is relatively large compared with that of the mouse. We aim to identify basic mechanisms that further the understanding of poor sight and blindness in humans and as models for treating or preventing these conditions.
  The objective of the second part of the project is to identify DNA markers (Single Nucleotide Polymorphisms- SNPs) for poultry breeders to use to improve the welfare of their stock. We also examine different aspects of the environment (e.g. factors causing foot pad dermatitis) so that short term changes can, if possible, be made to improve the welfare of commercial broilers and turkeys. In other research we aim to understand the genetic basis underlying the need for substantial feed restriction in broiler parent stock so that genetic selection can be performed that will result in rearing practices that optimise the welfare of the birds

- Outline the general project plan.

Where possible experiments are conducted on preserved tissues or in cell culture. Each experiment typically uses small numbers of birds or embryos (e.g. 3-6 per treatment group) and they are bred from 16 adults from each of the 4 lines. Similar numbers of normal controls are used. Other experiments are carried out on chicks from hatch to 6 weeks of age and may involve feeding a diet containing a drug to correct the missing protein (gene product) or wearing spectacles that obscure vision in one eye, typically for 7-14 days, and rarely for 5 weeks.

Experiments with broilers or turkeys are designed to investigate different management practices to maximise the welfare of broilers and turkeys. For example, evaluation of a feed ingredient to minimise litter moisture and foot pad dermatitis, or to prevent the development of skeletal disease.

Experiments are always conducted with the minimum number of animals that are necessary to achieve statistically significant results and with at least one control treatment.

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

The most common procedure carried out on live birds involves blood sampling for extraction of DNA. For many purposes a small drop of blood (7 µl) is sufficient and is obtained by a small prick in a wing vein. This minimises haematoma formation and is very quick compared with collection via a syringe. Adverse effects are minimal.

The induction of foot pad dermatitis, designed to investigate the causes and potential treatments for the condition commonly found in commercial flocks, causes a foot lesion to develop very rapidly (<7 days) and may be painful. Birds are closely monitored and individuals that show signs of pain when walking are immediately culled.

Feed restriction of broiler breeders may be used as is practiced in commercial flocks to understand the effects and consequences of this practice and to devise methods of overcoming the need for feed restriction.

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

Understanding development that is relevant to disease processes at the cellular and molecular level generates ideas that can be evaluated that will alleviate or prevent human and animal diseases. In our case our results will be relevant to diseases such as e.g. myopia, blindness, deafness, kidney failure, polydactyly (extra digits), recurrent respiratory problems and infertility).

Investigating disease processes that affect the welfare of commercial poultry (broilers, turkeys and ducks) will lead to improved management and genetic tools for improving the inherited health of the birds.

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

Some experiments are conducted on preserved tissues or in cell culture whereas other experiments are carried out on chicks from hatch to 6 weeks of age. Each experiment typically uses small numbers of embryos or chicks (e.g. 3-6 per treatment group) that are bred from 16 adults from each of the 4 lines. Similar numbers of normal control, unaffected birds are used. The number of birds used in experiments for studying the welfare of commercial poultry varies widely. For example, evaluation of different treatments for preventing foot pad dermatitis my use 100-200 birds; validating SNPs for a broiler breeder experiment may use 500-1000 but will likely involve merely collecting a small blood sample to obtain DNA. All experiments use the minimum number of embryos or birds to achieve statistically significant results.

All experiments use the minimum number of embryos or birds to allow differences of clinical significance to be detected as statistically significant with a power of at least 80%.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The mutations that we study are only present in chickens and the chick is a better models for the diseases that we investigate that the mouse. Where possible we use tissue from embryos, cell culture techniques and tissues from animals that have been killed by a schedule 1 method.

- Explain why the protocols and the way they are carried out should involve the least suffering.

  Every procedure is designed to minimise suffering. Most of the research involves *in vitro* studies (e.g. microscopy, proteomics and other laboratory techniques) or cell culture methods. Whereas we also investigate lesions that are very common in commercial broiler chickens and turkeys, careful and precisely defined end points are agreed with named vets to ensure that any potential suffering is minimised.