HOME OFFICE LICENSING

Since the last meeting, the ERP Certificate Holder's Advisory Group has recommended four applications for amendments to existing project licences and three applications for continuation project licences. The Committee is asked to receive and note these applications, which were:

Applications:

Project Title: **Innate Immune Mechanisms in Cardiovascular Disease**

An application to continue work from an existing Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

Project Title: **Modelling breast cancer biology and therapy in mouse**

An application to continue work from an existing Project Licence. The application is currently with the Home Office, and the project abstract is reproduced below for further information.

Project Title: **Function of proteins involved in human diseases**

An application to continue work from an existing Project Licence. The application is currently with the Home Office, and the project abstract is reproduced below for further information.

Amendments:

Project Title: **Study of Retinal Damage in Experimental Glaucoma**

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

Project Title: **Antibodies, blood products and tissues on a service basis**

An application to amend an existing Project Licence. The application is currently with the Home Office, and the lay summary is reproduced below for further information.

Project Title: **The genetic control of cancer**

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

Project Title: **Epigenetic coding of life experiences in the brain**

An application to amend an existing Project Licence. The application is currently with the Home Office, and the lay summary is reproduced below for further information.

BSO
16th March 2011

Lay Summary and Abstracts from Applications Processed by CHAG

Please note – These are for information only – no assessment by the Committee is needed

Application 1: Innate Immune Mechanisms in Cardiovascular Disease

Abstract: Heart disease is one of the leading causes of death in the UK, accounting for nearly 40% of all deaths annually. This project seeks to examine the role that the immune system plays in the progression of heart disease.

The innate immune system provides a rapid response to infection, however two key components of innate immunity, the **Complement** system and the immune signalling molecules known as **Cytokines** have been implicated in the development of atherosclerosis, the key process underlying heart disease.

We propose to examine the mechanisms behind Complement and Cytokine involvement in atherosclerosis. We will use the knowledge so gained to devise and test putative therapeutic agents in the apolipoprotein E knockout mouse model of atherosclerosis.

These twin programmes of work will use genetically engineered mice lacking various key cytokines or parts of the complement system. These mice (together with controls) will be fed a high fat diet for various time periods up to 12 months. At specific times mice will be humanely euthanized and their hearts and blood vessels examined and compared for evidence and extent of heart disease. In some cases, agents will be given, either to further elucidate key mechanisms or to test possible new therapeutics and ascertain whether they could be of any use in human heart disease.

It is currently impossible to carry out these studies *in vitro* because the complex immune, cellular and biochemical interactions that occur between the different cell types within the artery wall and the atherosclerotic plaque simply cannot be modelled in isolated cells. Thus if any new or improved therapies are to be developed to counter this major disease we believe that there are no alternatives to the use of this and other animal models.

To minimize animal usage, we utilise cell culture models, including foam cells derived from human and mouse macrophages, to help guide the design of animal experiments, ensuring sharp focus on key questions. Such *in vitro* models are also excellent testing grounds for putative new therapeutics, enabling us to proceed to animal studies only with the most effective candidates. Our work will use the well established apolipoprotein E mouse model of heart disease.

The key part of the protocols used in this project is the high fat feeding of experimental animals to induce atherosclerosis. The only side effect that occurs is the possibility of skin irritation after several months of fat feeding. Most of our experiments will finish before this becomes a problem; however, the small number of mice which may develop ulcers from these effects will be euthanized using a humane method. Discomfort deriving from other procedures such as administration of therapeutics will be minimised by good animal handling and appropriate use of anaesthetics.

Heart disease remains one of the major killers in the Western world and is rapidly becoming a health issue in developing countries. By learning how our own immune system contributes to this disease we increase the likelihood of being able to develop new and improved therapies to combat it.

Application 2: Modelling breast cancer biology and therapy in mouse

Abstract: The main aim of this project is to identify the molecular processes that control the expansion of cell numbers within the mouse mammary gland. We aim to demonstrate the importance of these processes for normal mammary function and for the development of mammary cancer. The expected outcome of this work will be the identification of candidate genes for use in diagnosis and treatment of breast cancer.

The project will involve the modification of individual genes within mouse mammary tissues or tumours, and observing their effects on mammary function and the incidence or progression of cancer. This will be performed in two ways: using gene modification techniques to introduce specific gene mutations into the genomes of mice; and genetically manipulating mammary cells grown in the laboratory in cell culture prior to re-introduction of these cells back into the mammary glands of recipient mice. Using existing mouse strains that are genetically predisposed to acquiring mammary tumours we will also test therapeutic and diagnostic strategies involving the same genes modified in the experiments outlined above.

A minority of the mice in this program of work will therefore acquire mammary and secondary tumours during their lifetime. The majority of experiments will be geared towards prevention of tumour development in these mice, however in some instances where characterisation of gene function is required, tumour progression may be accelerated. In either case, tumour size will not be permitted to exceed stipulated size limits, and morbidity will be carefully monitored, in order to minimise stress to the animals. Other adverse effects may include altered mammary gland function (e.g. Failure to lactate) which although may result in dams being unable to rear their pups, usually does not adversely affect the welfare of the affected animal. A proportion of experimental animals will also be subjected to discomfort following superficial surgery or during injections. This will be minimised with analgesics and/or anaesthetics where appropriate. Conditional transgenics will be used where possible to limit genetic modification to the mammary tissues of the adult. This refinement will minimize detrimental impact of mutations on the animals.

All aspects of this work will be supported by experiments involving cells isolated from mammary tissues and tumours and maintained in culture in the laboratory. This will reduce the number of animals required. However animals are required to study tumour biology because it involves a complex interaction between multiple tissue types and the immune system, therefore analysis of tumour behaviour ultimately requires intervention in the context of the whole organism. The use of non-invasive imaging techniques, while part of the research and development of new diagnostic tools in their own right, will further reduce animal numbers.

Mice are considered the most suitable experimental animal model for breast cancer for the following reasons: there is a large body of knowledge on the physiology, histology and molecular biology of the mouse mammary gland; there are a wide range of existing genetically modified lines, a number of which are directly relevant and amenable to our studies; the relatively short life-cycle and high fecundity of mice is advantageous for genetic studies; and mice are generally regarded as being of lower sentience compared to other mammals such as the primates.

Application 3: Function of proteins involved in human diseases

Abstract: The project is devoted to studies of normal function and dysfunction of certain proteins and intracellular molecular pathways implicated in aetiology and pathogenesis of human diseases, primarily neurodegenerative diseases.

Neurodegenerative diseases are among the main causes of disability in elderly people. With the number of people suffering from these diseases in the developed countries growing sharply, they have become one of the most serious health problems. This is putting an increasing strain on our already overstretched health system and also on the budget and life stile of patients' families who take the largest slice of the care burden. Population modeling studies show that delaying the onset of neurodegenerative diseases by several years could decrease their prevalence in the middle of the century by approximately one third.
In this project we will search for new molecular and cellular targets that could be used for therapy of motor neuron, Alzheimer's and Parkinson's diseases. We will also assess efficiency and reveal mechanism of action of certain new potentially therapeutic compounds.
To achieve the goals of the project we will carry out detailed studies of previously generated mice with specific alterations of the genes of interest and new genetically altered mouse lines that we will produce. These animals develop various changes in their nervous system that model changes associated with neurodegeneration in human diseases. Combination of behavioral, morphological, physiological and biochemical experimental techniques will allow us to reveal how particular functional changes of genes and encoded proteins affect the nervous system of experimental animals and to draw correlations with available clinical data. Mechanism of action of new drugs will be assessed by testing their effects on animals that model specific types and steps of neurodegeneration.
Various in vitro and ex vivo approaches that we and other researchers use to study neurodegenerative processes give only limited information about specific molecular and cellular events that take place in degenerating nervous system but precise understanding of the disease development and progression requires systemic studies of the neural functions that could be carried out only in the context of the whole organism. Mouse models with modified expression of disease-associated genes are central for these studies because certain types of genetic manipulations could be practically done only in mice.
We will design our experiments to ensure that the minimal number of animals will be used in the project; wherever possible the same animals will be continually used to achieve different experimental endpoints. Most of genetic alterations that will be studied in the project cause only mild changes to mouse physiology that do not instigate significant problems to the health of juvenile or adult animals. However some ageing animals or animals treated with specific (e.g. neurotoxic) agents might develop adverse phenotype (e.g. muscle weakness, coordination loss)

and in these cases we will implement specific protocols to minimise animal suffering.
We believe that results of proposed studies will provide the valuable information required for development of new drugs, which will be able to slow down, or even prevent, various neurodegenerative diseases.

Amendment 1: Study of Retinal Damage in Experimental Glaucoma

1. Lay Summary: What the changes are:

Changes have been made to protocol 1 of this license to include the means to make electrophysiological recordings from the visual centres of rats and tree shrews. The animals will be maintained as paralysed anaesthetised preparations.

The following has been added to protocol 1.

Technique 10: Electrophysiological assessment of experimental glaucoma by invasive electrophysiology:
Expected adverse effects: local inflammation (1 in 5)

Technique 11: In vivo labelling of retinal ganglion cell:
Expected adverse effects: Local inflammation (1 in 20)

2. Why the changes are needed:

In our work with the rodent model of experimental glaucoma, we have evidence that it may be possible to recover retinal ganglion cell (RGC) structure by manipulating the neuronal environment that supports these cells.)By reducing the intraocular pressure and digesting the perineuronal net (PNN0 that surrounds these cells we have observed partial but very promising recovery in RGC structure. We now want to see if these changes result in any improvement in RGC function. To do this, we need to record from visual centres that receive input from these cells in our rodent glaucoma model.

3. Species and number to be used

- We have asked for an additional 100 rats over the course of the project license to bring the total to 400. These additional animals reflect the numbers needed to assess the effect of PNN digestion and to ensure that this treatment in itself is not harmful. These animals will also be used in electrophysiological studies.

4. Effects on the animals:

Technique 10 specifies the use of electrophysiological recording techniques to monitor the visual inputs to the CNS. Animals will be anaesthetised and subjected to neuromuscular blockade. All experiments will end with termination of the animal and the level of anaesthesia will be continuously monitored throughout the experiments by EEG and ECG. All animals will be artificially ventilated once the NMBA has been administered.
Technique 11 specifies the use of agents to label retinal ganglion cells in vivo. We will use nanoparticles which can be directed under an external magnetic

field to label the inner retina. Intraocular injections are well tolerated and are unlikely to lead to any significant suffering (severity: mild). This particulate labelling method will allow us to follow cells over time and, at the single cell level determine the beneficial effects of PNN disruption.

5. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

The additional 100 animals will experience minimal suffering in relation to the electrophysiological recordings since they will all be anesthetised prior to preparation for recordings and during the recording process. Animals receiving intravitreal injections will also undergo minimal suffering. A general anaesthetic will be administered for the injections. The injections are not expected to compromise visual performance.

b) Potential benefits:

The additional experiments will be crucial to achieving the objectives 3 and 4 set out in the project license. The electrophysiological studies are essential if we are establish that PNN modification can result in RGC recovery.

c) How the benefits outweigh the costs:

These additional experiments are essential in the development of novel therapeutic interventions targeting recovery of the visual system in glaucoma. We need first to establish that the improvements in retinal ganglion cell structure and function are robust and second to rule out the possibility that PNN disrupting agents can have long term detrimental effects on retinal function.

Amendment 2: Antibodies, blood products and tissues on a service basis

1. Lay Summary: What the changes are:

Additional protocol 19b 5 to allow a pre-treatment injection of an anti-coagulant in preparation for cardiac myocyte isolation.

2. Why the changes are needed:

A group within the department of Cardiology are interested in the relationship between structure and function of ion channels in physiological and patho-physiological states. They especially focus their investigations on the calcium release channel known as ryanodine receptor (RyR). The latter is the key protein of the excitation-contraction coupling in the heart, and as a regulator of calcium release, it is involved in pivotal functions such as development, apoptosis, secretion and gene expression.
Alterations of RyR in terms of structure and/or function lead to cardiac diseases such as heart failure and arrhythmias. RyR is therefore increasingly becoming a promising potential therapeutic target.

To test the effect of drugs on RyR, they propose a range of assays based on molecular biology and biochemistry techniques, as well as electrophysiology functional assays. However they now want to establish an assay that would represent a more "physiological" system. The most reliable assay is the use of adult cardiac myocytes from animals.
Alternative systems were recently developed to help reduce the use of animals (embryonic cell lines, stem cell-derived myocytes, computer modelling), none of these methods can supply cells that resemble the genotype and phenotype of adult cardiac myocytes well enough. And in order to screen potent drugs acting on adult pathologies, it is extremely important to be able to rely upon a valid system.

3. Species and number to be used

The rat has been chosen because the isolation of cardiac myocytes from rats and the characterization of their properties have been well established for many years. Furthermore they have a huge experience in the use of isolated cardiac myocytes from rats and related results have been published in international peer reviewed journals.

- The additional procedure of a pre-treatment IP injection in preparation for cardiac myocyte isolation in Protocol 5 to be applied to 150 adult rats.

4. Effects on the animals:

Adult male Wistar rats (6 to 12 weeks old, 200-400 g) will receive a pre-treatment of and anti-coagulant.
Rats are injected (intraperitoneal injection) with 500-1000 µl (depending on the body weight) of a citrate (40 mM, 117 mg Na-citrate in 10 ml 0.9% NaCl) solution for the prevention of blood clot formation.
Alternatively rats are injected (intraperitoneal injection) with Na-heparin (100-200 units/100 g body weight, (intraperitoneal injection).
Ten minutes later, the animal is terminated by a schedule 1 method.
The dose levels to be administered should produce no toxic effect to the animals. Only transient pain at the point of injection is expected.
The protocol has been successfully used by the group and has also been published in international peer reviewed journals. The IP injection route does not alter the physiology of the animal and the 10 minute interval between the injection and cull has produced successful isolation of cardiac myocytes. To avoid failure of the injection, the rats will be restrained in such a manner to expose the right hand side of the lower abdomen to the experienced technician performing the injection. The rat will also be held with its head lowered to aid displacement of organs from the injection site. A fine, short needle will be used.
The subcutaneous injection route has been considered but it is unclear what effects the slower absolution rate has on the results and if the technique alters the physiology.
All procedures will be conducted by well-trained and experienced licensed animal technicians with the ability to handle animals sympathetically.

5. Brief summary of the cost-benefit ratio of the proposal:

d) Costs to the animals:

150 rats will experience only transient discomfort from intraperitoneal injections on no more than one occasions.

e) Potential benefits:

This work will provide the Cardiology department with the data to explore the relationship between structure and function of ion channels in physiological and patho-physiological states.

f) How the benefits outweigh the costs:

The non control of the coagulation state in the freshly killed rat leads to a great variability in the quality of the isolated heart and therefore to a great variability in the quality of cardiac myocytes required. This great variability is characterised by a frequent low yield in available myocytes. As a consequence, more animals are required to get a viable preparation of cardiac cells:
Preliminary results obtained here in Cardiff showed that at least 2/3 of the rats killed without an injection of citrate presented a strong coagulation (regardless of age and weight) leading to the formation of blood clots in the heart immediately after the killing. Subsequent injections of citrate directly into the heart (after the animals were confirmed dead) were useless. These hearts were perfused with washing buffers and collagenase solutions, but any perfusion was greatly compromised by the presence of these blood clots. The evidence for that was simply the low yield of viable myocytes at the end of the isolation procedure.
In order to avoid this waste of animals, time and money, it is essential to control the formation of blood clots. To achieve this, the most appropriate way is to inject an anticoagulant solution (citrate or heparin) before the sacrifice of the animal.
It is ethically, scientifically and economically relevant to add this protocol to this licence as all procedures will be carried out at a highly professional standard by well-trained and experienced licensed animal technicians, with the ability to handle animals sympathetically. The NVS and NACWO will assist and advise if any unpredicted adverse effects occur.

Amendment 3: The genetic control of cancer

1. **Lay Summary: What the changes are:**
   Adjustments to protocols 19b 7 and 8 to allow administration of substances via slow release into the circulation over a prolonged period of time.

2. **Why the changes are needed:**
   A new collaboration permits us to explore the potential therapeutic role of a novel vaccine for use in colorectal cancer. This approach requires release of a long acting form of a substance as part of the treatment regimen. This necessitates incorporation of a new method for substance administration to address existing objectives.

3. **Species and number to be used**
   The additional procedure of surgical placement of long acting-depot substances in Protocol 7 and 8 will be applied to 50 adult mice as continued use from existing protocols.

4. **Effects on the animals:**
   Surgically-placed depot preparations/substances (e.g. in the form of pellets) will be placed aseptically under the skin via a small skin incision. This defect will then be repaired (e.g. by absorbable suture or tissue glue or as deemed necessary by the named veterinary surgeon) whilst the animal remains under general anaesthesia. Surgical placement of substances will be done under aseptic conditions to avoid infection, but veterinary advice will be sought in the event of wound infection. Mice will be closely monitored in the post-operative period to ensure infection is detected early. Suturing will be re-fashioned in the event of failure on one occasion only, and veterinary advice will be sought if there is further failure. The likelihood of long lasting harm from this procedure is very small. Depot preparations are frequently administered to patients without harm.

5. **Brief summary of the cost-benefit ratio of the proposal:**

   g) Costs to the animals:
   50 adult mice will experience transient discomfort from the insertion of substances under the skin. Administration may need to be repeated dependant upon the drug and the timing if its effects. If repeat administration is required the advice of the named veterinary surgeon will be sought to minimise harm.

   h) Potential benefits:
   This work will provide additional data to address objective 3 of the PPL, thus providing a valuable contribution towards the development and testing of new therapeutic strategies in colon cancer.

   i) How the benefits outweigh the costs:

The important additional knowledge gained by developing new therapies in the context of animal models has the potential to influence early phase clinical trial design and improve the care received by cancer patients.
The use of long acting substances avoids repeated administration and any associated distress. This represents a refinement in technique in keeping with the 3R's. All animals will be closely monitored during experiments by those responsible and animal care advisors, ensuring unnecessary suffering is minimised. The experimental approach will be discussed with the named animal care worker from the outset to ensure familiarity with the procedure, possible adverse effects (section 4) and aims of the research. The assistance of the named animal worker during placement of pellets, although not required, would be encouraged. The advice of the named veterinary surgeon will be sought as necessary (section 4).

Amendment 4: Epigenetic coding of life experiences in the brain

1. Lay Summary: What the changes are:

New paragraph Section 17 p.3

New references included in reference list

New sentence inserted Section 18 p.3

Protocol 19b1 ('Breeding and maintenance of genetically altered mice')split into similar protocols (19b1A and 19b1B) to reflect 'mild' and 'moderate' severity GMO mouse lines. This change is reflected in section 19a, the list of protocols.

Inclusion of 'hair sampling' for genotyping in section v of the 19b1 protocols.

Appendices 5 and 6 added.

2. Why the changes are needed:

This project licence is focused on how epigenetic mechanisms may encode life events at the molecular level. Very recently work has suggested that imprinted genes, which are subject to a high degree of epigenetic regulation normally, are as a consequence especially vulnerable to altered environments and life events (*1*). This is particularly so for imprinted genes expressed in the brain (*2, 3*).

Consequently I would like to add mouse models in which imprinted gene expression has been altered to my licence. This is the main reason for making an amendment to the licence, and is the rationale for the inserted text in Sections 17 and 18; the additional reference; the splitting of the protocol 19b1; and the additional appendices. There was always the intention of including mouse work on this licence, and this amendment is necessary to indicate the mouse lines we propose to use.

In addition I have also added 'hair sampling' to the list of methods to extract DNA samples for genotyping. This is a more humane way of sampling than either ear-punch methods or tail-tipping (*4*).

1. C. Gallou-Kabani *et al.*, Sex- and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. *PLoS ONE* **5**, e14398 (2010).
2. M. J. Meaney, A. C. Ferguson-Smith, Epigenetic regulation of the neural transcriptome: the meaning of the marks. *Nature neuroscience* **13**, 1313 (Nov, 2010).
3. Z. Vucetic *et al.*, Early life protein restriction alters dopamine circuitry. *Neuroscience* **168**, 359 (Jun 30, 2010).
4. E. M. Schmitteckert, C. M. Prokop, H. J. Hedrich, DNA detection in hair of transgenic mice--a simple technique minimizing the distress on the animals. *Lab Anim* **33**, 385 (Oct, 1999).

3. Species and number to be used

700 additional adult mice in total to be used over the remaining 3.5 years of the licence.

4. Effects on the animals:

There are no changes in procedures (apart from the division of 19b1 into A and B to reflect mild and moderate genetically modified lines.

5. Brief summary of the cost-benefit ratio of the proposal:

j) Costs to the animals:

Increase in mouse numbers. Some of these will be classed as moderate in terms of breeding.

k) Potential benefits:

This work will provide a novel angle for investigation and additional data to address Objective 2 of the PPL e.g. "To characterise the epigenetic changes that are associated with behavioural changes due to altered socialisation at key stages of development"

l) How the benefits outweigh the costs:

There are no new procedures in this amendment, only the addition of mouse lines (and an increase in numbers) and the opening of a new angle in which to explore the question addressed in this PPL. This new line of enquiry may point directly to genes that are vulnerable to adverse environment and life events. This will increase our understanding of how such life events can influence brain and behaviour, and will possibly give rise to a greater understanding how life events can lead to debilitating episodes of mental illness such as anxiety, drug addiction, stress, and depression.