HOME OFFICE LICENSING

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

Applications:

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

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: Imaging Dendritic Function in Cerebral Cortex

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: Tumour Therapy in Tuberous Sclerosis Complex

An application to amend an existing Project Licence. The application has completed ERP and is being prepared for Home Office submission, and the lay summary is reproduced below for further information.

Project Title: **Cell Signalling in Development and Disease**

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: **Immune Cell migration in Health and Disease**

An application to amend an existing Project Licence, dealt with by fast-track ERP. The application has completed ERP and is being prepared for Home Office submission, and the lay summary is reproduced below for further information.

Project Title: **Modelling Neuroendocrine and Metabolic Function**

An application to amend an existing Project Licence. The application has completed ERP and is being prepared for Home Office submission, and the lay summary is reproduced below for further information.

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

An application to amend an existing Project Licence. The application has completed ERP and is being prepared for Home Office submission, and the lay summary is reproduced below for further information.

BSO
10 March 2012

LAY SUMMARY AND ABSTRACTS FROM APPLICATIONS PROCESSED BY CHAG

Please NOTE – This is for information only – no assessment by the Committee is needed

Application 1 – Antibodies, blood product and tissues on a service basis

Abstract. This service licence will provide centralised, expert assistance to various scientific research programmes with a need to use laboratory species for the production of both monoclonal and polyclonal antibodies, normal blood, its constituent components and tissue. Such biological material can play a key role in the investigation of disease and development of early diagnosis strategies.

Antibodies are naturally produced by an animal's immune system in response to a challenge to foreign material commonly called the antigen or immunogen. Introduction of the antigen by injection using common routes and minimal volumes ensures there is little or no discomfort to the animals. The antibodies required for the research programmes are typically found in the blood of the immunised animals and this is measured as the titre. Small blood samples are taken and analgesia is often used to ensure the animals suffer only minimal levels of discomfort. Care is also taken to ensure there is little opportunity for infection during injection or sampling. At all times animals undergo regular health checks and on the rare occasion of any showing signs of illness, prompt action is taken to alleviate any pain and veterinary advice is sought. Mice will be the main species of choice for monoclonal production but rats may also be used. Polyclonal antibodies will normally be produced in rabbits, however rats and guinea pigs may also be used where they are deemed the most appropriate to produce a high-quality end-result.

For the Pre-treatment protocol, animals will be used as a fresh source of tissue. The tissue needs to be fresh in order to isolate highly viable and functional cells (the smallest functional/living part of any tissue). The isolated cells act as 'tiny organs' in their own right. As you can isolate so many cells from a single piece of tissue you can conduct a very large number of tests, thereby reducing the number of animals used.

For the preservation of differentiated primary cells, animals will be treated with hormones (e.g. glucocorticoids) and known inducers of the Cytochrome P450 system (e.g. Phenobarbitol, 3-Methylcholanthracene) prior to being killed. This procedure will allow the fate of the specialised cells and enzyme systems to be traced during subsequent *in vitro* culture. The cells are harvested and mixed with a unique protein complex. The protein complex 'stabilises' the cells and keeps them fresh (normally when out of the body they quickly deteriorate/die). The stabilised cells last for several (5) days. Being stabilised allows them to be shipped to many different researchers across the UK and Europe.

Cardiac myocyt cell isolation will enable research into Heart diseases. To understand how the adult heart can lose its function, it is necessary to study signalling mechanisms on the single-cell level. Therefore isolated cardiac muscle cells represent a stable and reliable model where each individual cell can be examined in terms of function and the cellular environment can be

controlled. Animals will be injected with an anti-coagulant prior to being killed. This procedure will prevent the formation of blood clots within the ventricular chambers, in order to reduce the amount of animals employed for the investigation.

Rats will be the main species of choice for pre-treatment and tissue harvest, however mice may also be used where they are deemed the most appropriate to produce a high-quality end-result.

The licence will be used where there is no non-animal alternative and proposals to utilise this centralised service will be scrutinised by the licence holder, NACWO, ODF, BSO. Persons engaged in the procedures are expert in the conduct of such procedures and through the centralisation of this service data will be used to identify optimal procedures to produce high quality end-results using minimal numbers of animals

Application 2 – Imaging Dendritic Function in Cerebral Cortex

Abstract. This project will explore the information processing, decision-making and short-term memory capabilities of brain cells, dendrites (branches), and synapses in the cerebral cortex, using fluorescent indicators, microscopy, electrical recording and complementary techniques. We will also investigate how neurons, dendrites and synapses 'go wrong' in animal models of brain diseases such as schizophrenia. In order to do this, we will breed and maintain normal and genetically-modified rats and mice. Experiments will only be performed on brain slices or fully anaesthetised rats/mice.

In brain slices, we will measure the sizes of synaptic inputs, explore the computational capabilities of dendrites by stimulating groups of synapses to fire 'NMDA spikes', and test whether longer-lasting stimuli can convert single dendrites into bistable devices, which can retain a 'memory' for several seconds. We will characterise the 'flashes' during NMDA spikes and 'up' states in neurons containing fluorescent indicators. We will look for similar events in anaesthetised rats/mice, including during sensory stimulation.

Schizophrenia involves under-function or loss of excitatory synapses, contributing to deficits in seconds-long memory retention. We will repeat the experiments above, looking for abnormalities, in rats or mice mimicking important aspects of schizophrenia and related disorders.

Computer simulations will be carried out to pre-test biological experiments, reducing animal use - although usually there are too many unknowns for firm conclusions beyond proof-of-principle. Experiments on real brain tissue are therefore vital. Neuronal cultures have abnormal neurons and circuitry. Brain slices are much closer to normal, so are suitable for many of our questions. However, to discover whether dendritic NMDA spikes or bistability occur in normal brains, we need whole-animal experiments.

Brain slices are far too small to be conscious. Their preparation involves no pain; the rats or mice are given deep general anaesthesia, during which the brain is removed and cooled to a temperature at which no nerve impulses occur, before

slicing. Whole-animal experiments will be performed completely under general anaesthesia. Animals will be monitored closely, never wake up, experience no discomfort and will be humanely killed at the end. Tissue samples may be taken.

Genetically-modified animals may have tiny holes clipped in their ears for identification and gene checking, causing mild momentary discomfort. Some animals may experience mild discomfort as a result of their genetic alterations. This will be carefully monitored, and minimised.

A few hundred rats and mice will be needed as several kinds of difficult and complex experiment are planned; the results must be reproduced. Rats and mice are ideal: their brains are small for mammals, have essentially the same components/circuitry as humans, but far fewer neurons. A large amount is known about them, they are easy to look after, they breed fast, strains with uniform genetic background are available (reducing variability, hence animal numbers required). A wide variety of strains are available with abnormal gene variants linked to mental illness.

The benefits of this research include a better understanding of brain function, and insights into common diseases such as schizophrenia and epilepsy which cause immense suffering and economic loss.

Amendment 1 - Tumour Therapy in Tuberous Sclerosis Complex

Lay Summary:

1. What the changes are:

a. Modifications to allow more techniques to be used for in vivo imaging and to allow early start of treatment and long duration of treatment.

b. Modifications of protocol 19b 1 to allow more techniques to be used for collecting blood samples.

c. Modification of protocol 19b 2 to allow more techniques to be used for in vivo imaging, to classify oral and gavage administration of agents, and to allow early start of treatment and long duration of treatment.

2. Why the changes are needed:

a. We have added a sub-section in Section 18b: Amendment November 2011. As discussed in Section 17, accurate detection of lesions *in vivo* is essential to assessment of therapeutic effects of chemicals/drugs in mouse tumour models. In combination with MRI, recently available imaging techniques such as PET in the university may help to more accurately detect solid renal lesions in the mouse models. This may allow us to obtain more reliable data and in consequence to reduce the mouse number to be used.

We have changed treatment start age and duration. In order to test the therapeutic effect of therapeutic agents, we will start to treat animals at the age of 2 to 18 months and the treatment will continue for one week to life time. This is because we have found that the mouse models can develop tumours as early as 2 months of age and that the tumours re-grow when some of the agents used are withdrawn.

To test potential effects of agents on tumour prevention, we may start to treat animals as early as one week old. We may also treat pregnant mice to prevent tumour development in their offspring. This is because it is believed that the tumour initiation may occur very early in life. To treat animals as young as one week, intraperitoneal injection will be used for administration of agents. The preventive treatment may last for up to 24 months or life time because tumour initiation may occur at any time of life, particularly in these animal models, with an increase risk of tumorigenesis as animals get old.

b. We have changed the wording in protocol 19b 1 (5) "Blood samples will be taken by venopuncture of superficial vessels to determine plasma levels of growth factors" to "Blood samples will be taken from superficial vessels, e.g. tail vein, to determine plasma (serum) levels to determine plasma levels of growth factors". This will allow more flexibility and less severity whenever possible in techniques to be used.

c. We have modified protocol 19b 2 as follows and the reasons for the modifications are described as above (in a).

1. *In vivo* imaging (optional) Experimental animals and controls at various stages of disease process before and/or after administration of therapeutics will be injected intravenously (AB) with labelled protein tags or unlabelled chemicals suitable for imaging using MRI, PET, SPECT, CT or other imaging modalities and imaged once or multiple times between 0 and 30 minutes after injection; general anaesthesia will be maintained throughout the imaging procedure for a maximum duration of 90 minutes. For some MRI protocols, injection of labelled protein tags or unlabelled chemicals is not necessary. If animals with a transgenic fluorescence protein are used, injection of labelled protein tags may not be required for *in vivo* fluorescence imaging. Imaging may be repeated up to 10 times at intervals of no less than 7 days.

2. Test substances or vehicle alone will be delivered orally (including by gavage and in mixture with food and/or drinking water) or by intramuscular, subcutaneous, intravenous or intraperitoneal injection (AA/AB). Full dose administration of one to seven times a week may last one week to life time. For

the purpose of prevention, similar but in most cases lower dose (compared with full dose) administration may last up to life time. To treat animals as young as one week, intraperitoneal injection will normally be used for administration of agents.

3. Species and number to be used

For the modified protocol19b 2,

No additional adult mice

400 young mice (= or >1 week) to be added to the licence

4. Effects on the animals:

a. Advice has been taken on dosages and durations of imaging procedures required from s40 Personal who has long experience with *in vivo* nuclear imaging techniques. No adverse effects are anticipated from the administration of radiolabelled tracer substances at the doses and durations to be used. Animals will be monitored closely during the procedure (heart rate, temperature and cctv while under anaesthesias within the scanner) and on the appearance of any adverse effects the procedure will be terminated and the advice of the Named Animal Care and Welfare Officer (NACWO) and/or named veterinary surgeon (NVS) will be sought as appropriate.

b. Administration of substances by injection may cause infection but this extremely rare. The risk of infection will be minimised by good sterile techniques. Extra care will be taken to avoid administration into trachaea when gavage is used. When treating very young animals (e.g. 1 week old), no gavage will be used.

c. Substances administered should have little or no detrimental effect on the health of the animals. In some cases, effective doses have been described in details in literatures. On occasions when new agents are to be tested, stepwise tests will be used to start with low dose in no more than two animals each step. In rare events that animals show a debilitating reaction to the substance, e.g., rapid weight loss (note: Some anti-tumour chemicals may cause up to 20% of weight loss and this is acceptable only if general condition is good.), increased vocalisation and adverse behavioural changes, the treatment will be stopped immediately. If the animals do not show spontaneous recovery within the expected period of activity of the substance, they will be removed from the study immediately and humanely killed.

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

a) Costs to the animals:

Animals will experience some transient discomfort during preparation for in vivo imaging or from administration of agents by gavage, intramuscular, subcutaneous, intravenous or intraperitoneal injection.

b) Potential benefits:

This work will provide additional data to address Objectives 1 to 4 of the PPL and therefore help to identify more effective therapeutic and preventive agents and strategies for tuberous sclerosis associated lesions. It will also help to reveal mechanisms underlying drug action and resistance.

c) How the benefits outweigh the costs:

Possible adverse effects caused by the modified protocols can be minimised by the measures mentioned above. These modifications are very important for a number of reasons. Firstly, new in vivo imaging techniques would allow more accurate detection of renal lesions. This may allow us to obtain more reliable data and in consequence to reduce the mouse number to be used. Secondly, early and long treatment may allow identification of agents and strategies to prevent tumour initiation in early life of the mouse models. This could be directly translated to clinical benefit for patients. Finally, this work will no doubt help to understand mechanisms underlying drug action and resistance.

<u>Amendment 2</u> - Cell Signalling in Development and Disease

Lay Summary:

1. What the changes are:

Changes to protocols 7 and 8 to allow oral administration of potentially anticancer drug candidates to genetically-modified mice in a liquid form. There is no change to the number or type of mice to be used.

2. Why the changes are needed:

Due to the excellent progress made in ongoing work under s38 Health and Safety, s40 Personal Data the conditional mouse models we have developed have been selected for use in *in vivo* trials of small molecule inhibitors that are being developed with s40 Personal Data. These trials include short term studies of small molecule inhibitors on Wnt-modulated biomarkers of the small intestine and longer term studies on Wnt-driven tumour models. As the final goal of these studies is the development of an orally available small molecule inhibitor of the Wnt pathway (Objective 3), it is important that

these studies are carried out by oral administration of candidate drugs. This change will also constitute a refinement on the current approach in which animals are given repeated intraperitoneal injections. Prior to extensive research into drug formulation, the most effective route to administer these compounds will be through oral delivery of compounds in a liquid form.

3. Species and number to be used

The additional procedures in Protocols 7 and 8 would be applied to approximately 50% of the existing 3, 000 mice that have been estimated to be needed for Protocols 7 and 8. No total increase in mouse numbers is required.

4. Effects on the animals:

Oral administration of potentially therapeutic compounds using a gavage would be carried out twice daily (three times if the total period of exposure was 24 hours or less). Analogous studies carried out in other laboratories suggest that treatment may cause some loss of condition after 5 days. Any sign of loss of condition would lead to a reduction in the number of gavages given for that compound/dose.

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

d) Costs to the animals:

Mice will experience transient discomfort from oral gavage administration two to three times a day. Repeated oral compound administration by gavage may rarely result in some solution entering the trachea and lungs and result in breathing difficulty manifested by laboured breathing and gasping for breath. Animals showing signs of breathing difficulty will be killed by a schedule 1 method. Damage to the mouth and/or oesophagus resulting in haemorrhage may also occur. Unless this haemorrhage can be controlled the animal will be killed by a schedule 1 method.

e) Potential benefits:

Oral gavage compound administration experiments should determine if a potentially therapeutic compound that was identified by cell-based assays inhibits the Wnt pathway *in vivo*. This is an essential step in the ongoing development of anti-cancer drugs. Oral delivery of compound will also reduce the need for intra-peritoneal injection.

f) How the benefits outweigh the costs:

The expected achievement from this programme of work will be the development of one or more compounds that will be taken for phase 1 clinical trials as an anti-cancer agent in patients. The data that will be produced should show efficacy of the compound(s) in highly

relevant tumour models that recapitulate key aspects of the human disease. The conditional mouse models will allow high quality pharmacodynamic studies to be carried out to provide supporting data for the initiation of clinical trials. Oral administration is preferred over the intraperitoneal route for multiple administrations because of the possibility of complications resulting in peritonitis with the latter approach (see Diehl et al J. Appl. Toxicol. 21, 15-23). 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.

<u>Amendment 3</u> - Immune Cell migration in Health and Disease

Lay Summary:

1. What the changes are:

Changes to section 18b and a refinement to protocol 19b, 1 to enable measurement of tumour size by bioluminescent imaging or positron emission tomography.

2. Why the changes are needed:

The location of subcutaneous tumours needs to accessible for imaging and should not be restricted to a single site. The flank will be the site most commonly used for these studies.

3. Species and number to be used:

I am not requesting the use of additional animals for this amendment.

4. Effects on the animals:

Transplantable tumour cell lines, such as the B16F10 melanoma, have been administered subcutaneously to a number of different sites including leg, flank and dorsum for studies of the impact of the immune system on tumour growth in mice. Discomfort experienced by the animals will depend on both tumour size and location.

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

g) Costs to the animals:

Daily monitoring of tumour growth according to UKCCCR guidelines will ensure that discomfort is minimised.

h) Potential benefits:

The ability to detect tumours before they become palpable will allow immunotherapies to be initiated at an early stage thus reducing the overall tumour burden to individual mice.

i) How the benefits outweigh the costs:

The ability to detect tumours before they become palpable will allow immunotherapies to be initiated before tumours become too large to be effectively removed by the immune response. This will increase the number of mice that respond to T cell therapy and therefore reduce the number of mice used.

Amendment 4 - Modelling Neuroendocrine and Metabolic Function

1. Lay Summary: What the changes are:

I have made the following changes:

c) Amended Amendment 2 in section 17 (page 8)

d) Amended Objective 2b (Section 18 page 5)

e) Introduced a new sentence on maintenance at non-code of practice temperatures in breeding and maintenance protocols NM05A and NM05B

NOTE: Despite these changes the overall severity limit of the licence is NOT increased and remains at moderate.

2. Why the changes are needed:

The minor amendments arise from our novel findings on the role of brown fat function in regulating white fat deposition in PWS-IC mice and the emerging literature indicating that thermoneutrality in mice is 30°C (Reviewed in Overton JM, *Int J Obesity* **34**:553-558, 2010), ie. 7-11°C above code of practice housing conditions. Thus, under standard temperatures, brown adipose tissue is activated as part of a thermal stress response, and results in reduced fat deposition. This effect appears to be exaggerated in the PWS-IC mouse model for Prader-Willi Syndrome, leading to profound leanness, whereas children with this condition develop abdominal obesity. Thus, after taking advice from the HOI, I have included the option of housing mice at temperatures at or just below the thermoneutral zone for mice (30°C) in the breeding and maintenance protocols (NM05A/B).

3. Species and number to be used

This amendment will NOT increase the number or severity of procedures performed on MICE in the REMAINING 7 MONTHS of this licence.

4. Effects on the animals:

Maintaining wild-type and PWS-IC mice at ambient temperatures above the code of practice will suppress the non-shivering thermogenic stress response, thereby making more fat available for storage in white adipose tissue.

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

j) Costs to the animals:

Mice maintained at higher ambient temperatures may show increased fat deposition and even develop obesity.

k) Potential benefits:

This amendment will have THREE potential benefits:

f) It will reduce the impact of the thermogenic stress in mice brought about by code of practice housing conditions.

g) Reveal the reason why mouse models for PWS are lean, whereas humans with this condition are obese.

h) Indicate whether activation of brown adipose tissue in PWS patients will alleviate the accompanying obesity.

l) How the benefits outweigh the costs:

As a highly experienced in vivo scientist, s40 Personal Data will work in close consultation with s38 Health and Safety, s40 and the named veterinary surgeon to maintain the professional standard of animal care for which he has an unblemished reputation. This amendment will be introduced in consultation with these staff and closely monitored.

Understanding the function of brown adipose tissue in PWS will indicate simple changes in housing conditions that will significantly enhance the lives of the 1:15,000 children born with this condition. Indeed, the results gained from mice with this particular condition may lead to the development of novel approaches to the treatment of obesity

Amendment 5 - Study of Retinal Damage in Experimental Glaucoma

Lay Summary: COVERING LETTER IN LIEU OF LAY SUMMARY:

Dear ERP,

I would like to apply for an amendment to transfer my Project License to s40 Personal Data . As a consultant ophthalmologist and leader of a research group working on the pathophysiology of retinal ganglion cells she is in a better position to lead this work. In addition, my own research group is expanding and I realize that I will no longer be able to devote as much time to this project as I have in the past.

It has been a pleasure to be involved in this work and to see the work in Optometry on glaucoma and retinal ganglion cell disease expand and I am delighted that has agreed to hold the license.