Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 32

Project Titles and key words

- Drug Disposition

  Pharmacokinetics, Absorption, Distribution, Metabolism, Excretion

- Molecular and cellular regulation

  Xenopus, amphibian, retinoid, nerve formation

- A porcine model of pulmonary hypertension

  Pig, Pulmonary Hypertension

- Analysis of the hedgehog pathway in zebrafish

  Hedgehog signaling, Vascular endothelial growth factor, adrenomedullin, embryonic patterning

- Adhesion molecules as treatment for medulloblastoma?

  Cancer, brain, diagnosis, therapy, medulloblastoma

- Developing an implantable vagus appetite regulator

  Obesity, diabetes,

- Germ cell development and embryogenesis

  Chromosomes, Trisomy, Infertility, Germ cells, Embryogenesis

- Genetic control of early mammalian development

  Mouse, embryo, transgenic, development

- Novel Immunotherapeutic interventions for cancer

  Immunotherapy, solid cancer, localising agents,

- Orofacial development, homeostasis and repair

  Teeth, tissue repair, regeneration, salivary glands

Project Title (max. 50 characters)	Drug Disposition
Key Words (max. 5 words)	Pharmacokinetics, Absorption, Distribution, Metabolism, Excretion
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)[1]	<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[2]</td><td></td><td>No</td></tr></table>
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to determine the fate of substances within the body and understand the relationship between the drug level and its pharmacological effect. This is required to obtain the correct doses of a substance for further testing or clinical use. Selecting the correct dose level is important to avoid high toxic levels and to predict whether enough can be given safely to humans for it to work. Novel substances will be given to animals by normal clinical routes such as oral or intravenous, and samples of blood and other body fluids will be taken at different times after dosing. This information helps exclude unsuitable substances and avoid further use of animals. Data generated under this project licence is required by regulatory agencies (eg. FDA & EMEA) before a new medicine can be tested in human volunteers or patients.
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)?	Studies under this project will give the information required to be confident that a new drug would be safe and effective in human use. It will help eliminate unsuitable substances at an early stage of drug development and identify those promising new agents that have the potential to become new medicines.
What species and approximate numbers of animals do you expect to use over what period of time?	Estimated animal numbers over the 5 year duration: Mice 7000; Rats 4600

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse events are not expected on this project and the incidence is very low. All studies are initiated at a low dose of a test substance and then only increased in the absence of adverse effects. With novel substances there is always risk of unexpected effects and animals are closely monitored for the first hour after dosing then periodically thereafter. Any adverse effects observed are scored by severity and any serious adverse effects then initiate the humane culling of the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Due to the complexity of mammalian biology combined with the difficulties of studying humans directly, it is extremely difficult to predict a drugs disposition profile. Novel small molecule drugs arise from diverse chemical series with unknown absorption, distribution, metabolism and elimination profiles. In all cases, it is important to understand whether a drug's disposition profile is appropriate for the intended clinical use. The integrated use of a diverse range of DMPK techniques (*in silico*, *in vitro* and *in vivo*) is therefore required to predict human drug disposition and to ensure adequate exposure in pharmacology and toxicology studies (Carlile *et al.* 1997; Naritomi *et al.* 2001).
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental design of *in vivo* studies ensures the minimum numbers of animals are used to generate the required information. Sensitive and accurate analysis of small volume serial blood samples enables a complete time course to be obtained from one animal after dosing (as opposed to using one animal per time point). Rats may also be used up to 4 times (continued use), reducing the total number of animals required to generate the same information. Wherever possible *in vitro/in silico* data are used to exclude substances with poor drug like properties from *in vivo* testing.
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 and rats are used since they are considered to be the lowest suitable mammals on the evolutionary tree which have physiology similar to humans. Serial blood sampling from animals and wherever possible from indwelling venous cannulae is the most efficient and accurate means of generating pharmacokinetic data from limited individual animals while causing the minimum of distress.

	Sensitive analytical methods means that substance levels can be detected in small biological matrix volumes, minimising the number of animals and amount of matrix required to generate quality PK data. Blood, tissues and cells generated from this project have great utility in *in vitro* assays to exclude large numbers of substances in a high throughput manner with poor drug like properties prior to *in vivo* testing. Animal suffering is minimised by using competent personnel. Studies are started at a low dose of a test substance and then increased only in the absence of adverse effects. Any adverse effects observed are scored by severity and any serious adverse effects then initiate the humane culling of the animal. Appropriate pain relief is given to animals recovering from a surgical procedure.

Molecular and cellular regulation
Xenopus, amphibian, retinoid, nerve formation

- Summarise your project (1-2 sentences)

Retinoic acid (RA), a derivative of vitamin A, is required to promote the formation of nerve cells. The aim is to use the formation of embryonic primary nerve cells, which form at an extremely early stage in the development of the amphibian, Xenopus, as a model system to understand the basic biology that underpins this process.

- 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.

  How retinoic acid, a derivative of vitamin A, promotes nerve formation is not fully understood. Although the system consists of a fairly simple collection of components, enzymes that make RA, proteins that transport and break down RA and proteins that interact with DNA to change gene activity in response to RA, details of how the production of the components is regulated are incomplete. The objective of this project is to understand further how retinoid signalling is regulated.

  This is of basic importance in understanding how nerve cells form, but is also likely to be relevant to the differentiation of neural stem cells, a process that also involves RA. Understanding this process will support the use of stem cell based neural therapies.

- Outline the general project plan.

The aim of the project is to understand in more detail how vitamin A derivatives affect nerve cell development using Xenopus as a model organism. One approach is to look at how the production of proteins that mediate the activity of RA is regulated. Unlike many genes, whose mRNA is turned directly into protein, for the retinoid receptors there seems to be an additional level that regulates when the mRNA can be used. By introducing synthetic mRNA into Xenopus embryos we can follow the factors that determine when the protein is produced. Retinoid signalling in nerve cell development requires two related families of protein called the RARs and the RXRs. Both are regulated in this way but is the system integrated to co-ordinate the activity of these two proteins?

RA is highly active and as a consequence its production, transport and turnover are carefully controlled. There is already some understanding of the regulation of individual components but the aim here is to get an overall picture of how the component parts contribute to the system, in one model organism. Initially this will involve describing when and where each of the components is made, using in situ hybridisation to detect the activity of each gene during the stages of Xenopus development.

In those parts of the embryo where RA is not present, the RAR and RXR proteins have to be switched off to prevent any leakey, ‘off-target’ effects. This is achieved with a corepressor protein called SMRT that binds to the RAR and RXR proteins in the absence of RA and stops them from working. Like many genes in the vertebrate genome, SMRT can encode many different proteins by altering the way its final mRNA is put together, or spliced. The idea is that by producing a number of SMRT variants, the one gene can generate a range of SMRT proteins one of which is highly efficient at recognising and inhibiting RAR and RXR proteins. We have already experimentally manipulated the outcome of splicing in Xenopus embryos and shown that this affects nerve cell development, but the next stage is a more comprehensive analysis of the different

mRNA variants. Importantly the range of variants is highly conserved between frogs and man, so the observations made in Xenopus will have significance for mammalian nerve cell formation.

- 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.

There are two procedures to be applied to the animals in this project. The first involves injecting females of Xenopus laevis with hormone to induce egg laying. This fundamental procedure is widespread and is considered mild as the females rarely, if ever, suffer any adverse effects such as infection and odema and consequently are often re-used. Re-use is dependent on a cycle of assessment that considers the condition of the frog at regular intervals during the 3-month recovery period. Unfortunately, Xenopus tropicalis females more often show the adverse effects described above and also unexplained death in the days and weeks following this procedure, so if required, they will be used once only and routinely killed by a schedule 1 method following egg laying.

The second procedure is the generation and maintenance of transgenic Xenopus laevis, primarily using either the Gateway or the TALEN methods of genome manipulation. In most cases the embryo will only show adverse effects when the mutation is homozygous and in these cases the end-point of the experiment will be reached before the independent feeding stage and so the embryos are not subject to ASPA. Some lines will be maintained as heterozygotes and these will be monitored for adverse effects and killed by a schedule 1 method if the tadpoles show any distress or abnormal behaviour.

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

Retinoid signalling plays a major role in vertebrate development influencing axial patterning and nerve formation. It is also a factor that promotes the nerve cell differentiation of stem cells, a process that is considered to be of potential therapeutic benefit. Understanding how retinoid signalling is regulated at all steps in the signalling pathway from synthesis of the RA, production of the proteins that respond to the signal, to the decay of the signal will provide a basic advance in how we interpret this biological system.

- 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.

Just one Xenopus female can produce a large number of embryos, so not many animals are needed for each experiment. On average, two female frogs per week will generate sufficient embryos. For each transgenic line, around 50 screened tadpoles will be allowed to metamorphose and the subsequent frogs used for breeding purposes. The aim is to create in the order of 5-10 lines over 5 years. We will use predominantly Xenopus laevis as this model organism has been extensively studied, its developmental programme is known in detail and its genome has been sequenced. Unlike mice and chicks, Xenopus embryos are accessible from the earliest stages of development. A more limited number of Xenopus tropicalis (around 20 per year) will be used. Although the TALEN method has been shown to be effective in X. laevis, even though it is essentially tetraploid, there may be occasions when use of the diploid X.tropicalis provides a more efficient route.

Using amphibians, that produce large numbers of embryos, limits the number of animals to be used. By employing a screening process to detect the transgenic and manipulated animals we can maintain just those animals that are fit for purpose.

- 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 project examines retinoid signalling as a biological system and preliminary evidence suggests that the production of retinoid receptor protein depends on signals that pass between different parts of the embryo that would be difficult to replicate in an in-vitro system until the identity of the signal is known. When looking at the function of the genes involved, it is important to see how they affect the development of the whole embryo. When constructing the reporter transgenic lines, the idea is to use these to follow the outcome of retinoid signalling, again within the whole embryo as a biological system rather than in a reductionist approach on individual cell types. The merit of this lies in the fact that RA is a diffusible signal, similar to a hormone,that affects many tissues.

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

The first protocol involves the production of eggs and embryos from adult female Xenopus laevis. This is a mild protocol that involves the injection of chorionic gonadotrophin into the female to stimulate ovulation. In the vast majority of cases with Xenopus laevis, the female shows no adverse effects. For most experiments we will use X.laevis, but where the genetics demand, we will use X.tropicalis but not attempt re-use to minimise the chance of suffering post-laying.

In most cases the genetic manipulation of the embryos will result in heterozygotes that do not display a phenotype. There is a possibility, using the efficient TALEN method, that some homozygotes will be created directly, so embryos will be screened for adverse effects and terminated before the stage of independent feeding and so will not be subject to ASPA. As we are interested in the effects of the genes in early embryos, affected homozygotes generated by breeding recessive, unaffected heterozygotes will also be terminated before the stage of independent feeding.

A porcine model of pulmonary hypertension
Pig, Pulmonary Hypertension

- Summarise your project (1-2 sentences)

Pulmonary hypertension (high blood pressure in the lungs) is a devastating and debilitating disease; mean survival is 2.8 years without treatment and survivors suffer severe, life limiting shortness of breath and heart failure. We aim to create a model of pulmonary hypertension in a pig and try to treat it using electrical energy to cauterise the nerve bundles in the lung arteries.

- 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.

Pulmonary hypertension (PH) is a devastating disease that causes death and significant suffering among patients. The drugs currently used to treat this disease cost up to £300,000 per patient, per year and are only minimally effective. Over active nerves supplying the blood vessels of the lungs contribute to the high blood pressure in the lungs in patients with PH. Surgical resection of these nerves significantly reduces PH; however, open chest surgery carries high risk in patients with PH. A new catheter-based technique for cauterising nerves has recently become available. This procedure may make it possible to remove the nerve supply to the arteries of the lungs, as achieved by surgery, using minimally invasive techniques that are very similar to those required to diagnose PH. We will use a needle in the neck to deliver a radio-frequency catheter to the lung arteries to allow us to cauterise their nerve supply. This method is used clinically to cauterise renal arteries in patients with systemic hypertension and carries a very low complication rate(1 in 1000). If successful, this procedure could quickly result in a human clinical trial and the potential for a new treatment for a devastating disease with no current cure.

- Outline the general project plan.

We plan to create a model of PH in pigs using a drug that causes blood vessels to contract. Once we have established the high blood pressure in the lungs we will insert a long catheter electrode into the blood vessels supplying the lungs through a vein. We will then use radio frequency energy to target the nerve supply of the blood vessels. The effect of the treatment will be determined by the change in the blood pressure in the blood vessels supplying the lung.

- 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.

Procedures
The pig will be injected with a sedative before being anesthetised. Then tubes will be inserted into the circulation to monitor the blood pressure in the lungs. A drug will be given to create PH. The electrode catheter will be passed, and electrical impulses used to ‘cauterise’ the nerves in the blood vessel wall. The effect of this treatment will be determined by repeated measurement of the blood pressure in the lungs.

Adverse effects
The animal will be under general anaesthetic throughout the procedure, so discomfort to the animal should be minimal, and the animal will be killed under anaesthetic.

The injection of sedative and induction of general anaesthetic may cause discomfort. There is a very small risk of death from the general anaesthetic. The insertion of tubes into the blood vessels may cause bleeding, which will be treated appropriately at the time. The drug used to raise the blood pressure in the lungs may cause clotting of the blood. This will be observed for and treated as necessary. The electrode passed into the blood vessels may cause damage to, and bleeding from the blood vessels. All these side effects will be carefully looked for during the procedure.

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

Without treatment, PH has an average survival of 2.8 years. Patients with the disease suffer significant shortness of breath and heart failure. Current treatments are only minimally effective and costly; there is no current cure. The treatment we propose adapts currently available techniques (used to treat high blood pressure) to the treatment of PH. If successful these experiments may lead quickly to human trials, and potentially to significant clinical benefit. The development of a new treatment offers real hope of reduced symptoms, improved quality of life and possibly longer life for 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.

To establish this model, and develop our proof-of-concept studies on the effect of sympathetic denervation, on PH we expect to use 16 pigs. This number is based upon a calculation taking into account the measurements used and expected effect size observed in other published experiments. The pig is of a similar size and anatomy to humans, allowing the use of clinically available catheters for the experiments proposed. The model of pulmonary hypertension used is effective in a range of animals and is used in the pig as a gold standard for the testing of potential new drugs for the treatment of pulmonary hypertension. We aim to use the minimum number of pigs possible to achieve this aim.

- 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 pig is physically, biologically and genetically similar to man. The complex physiology of the heart and lungs cannot be replicated in non-animal alternative and pre-clinical studies are required to test the effect of the treatment prior to human trials.

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

The protocols will cause minimal suffering to animals as they will be entirely performed under general anaesthetic.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Analysis of the Hedgehog pathway in zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hedgehog signaling, Vascular endothelial growth factor, adrenomedullin, embryonic patterning</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)[3]</td><td><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></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></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>An intercellular signal curiously named “hedgehog” is at the base of the most common form of cancer in human; basal cell carcinoma. It is one of a handful of signals that patterns the vertebrate embryo. How the signal is made and transmitted to the nucleus of the receiving cell is unclear, our work will help to clarify this. It is also not known precisely how further signals are activated by Hedgehog and how these signals (VEGF and Adrenomedullin, others) “talk back” to the hedgehog signal. Adrenomedullin and Hedgehog may also help to bypass the requirement for VEGF during bloodvessel formation, detailed understanding of this process may help to find better ways to block tumor growth.</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>We will produce data in fish that will clarify which genes act in the hedgehog pathway and how. This may be a paradigm for hedgehog signalling in higher vertebrates, including human. If we can prove that hedgehog and adrenomedullin can bypass VEGF during blood vessel patterning, this is clinically very important as it might be one of the reasons for the disappointing results of anti-VEGF therapy in cancer treatment. In this case combining anti-VEGF therapy with blockers of the other two pathways might improve outcome.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Over five years we estimate that we will use 19000 adult zebrafish, these are generally only used for matings. These numbers are determined by stock keeping requirements, a minimal number is require</td></tr>
</table>

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

over what period of time?	per stock to ensure maintenance or generation of the a required transgenic line, unwanted lines will usually be maintained as a frozen sperm sample. Maximally 4000 of these will be used to generate transgenic lines, the rest are required to maintain existing stocks. We will use maximally 1000 8 day old larvae for gene and protein expression studies, to study processes that occur later than d5. We will use a small number of adults to study behaviour of mutant cells in adults (80) or to create females which produce eggs that are deficient of a certain gene product (200). Over 5 years we will also use 200 fish where will inject drugs that activate of block the hedgehog pathway to study its role in adult tissues, focusing on effects on proliferation.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most cases we will work on embryos, therefore adult fish are only required in natural matings to generate these embryos. Occasionally to identify the correct genetic type of animal we will take a small part of the fin, this will regenerate and does not significantly impair their well being. We may raise embryos to adulthood that have been injected with DNA, RNA of hedgehog modulating compounds, or carry a number of genetically modified cells in their body. This is not expected to cause harm but unanticipated dominant effects may occur. In some cases fish may be anaesthetised required for eg. fin clips, observation, or expression of oocytes. Anaesthesia may cause light transient bleeding from their gills. Rarely fish may have difficulty waking up or very rarely they may fail to recover at all. At the end of a procedure (or their breeding life in case of breeding stock) animals will be euthanised
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying the role of hedgehog signalling, using zebrafish as our model organism, this needs to be studied in a living embryos/larvae because these processes occur as a consequence of a precise interplay between different tissues that send and receive patterning signals. For instance, hedgehog signalling from the midline notochord to the muscle precursors is important to induce VEGF in these cells, which in turn signals to the endothelial cells to induce the correct type of blood vessel. Such conditions are impossible to recreate using cell culture systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimal number of animals needed to keep the required lines, or to obtain statistically significant results. Importantly, most of our experiments are in fact done on embryos which are below the age of protection.

3. Refinement	
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.	Mating is a natural behaviour and is unlikely to cause harm. All other experimental procedures are carried out under anaesthesia and are only causing minimal harm to the larvae/adults, if for any reason a fish shows significant health problems, it will be euthanised immediately to prevent unnecessary suffering. Fish are the simplest vertebrate model system in which we can study the Hedgehog signal

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Adhesion molecules as treatment for medulloblastoma?</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, brain, diagnosis, therapy, medulloblastoma</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[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>Medulloblastoma is the most common malignant brain tumor of childhood, affecting 1:150,000 children with peak incidence at age five. Surgery, radiotherapy, and chemotherapy successfully cure many patients, but survivors can suffer long-term toxicities affecting their growth potential and mental abilities; furthermore, in up to 30% of cases there is no cure, reflecting our incomplete understanding of the underlying molecular and cellular processes. Our studies of these processes during normal development identified a naturally-occurring protein that stops the proliferation of a specific set of brain cells once their normal growth is complete. Since we know these cells can also grow abnormally to cause medulloblastoma, our objective here is to test whether this protein can also stop this abnormal growth. If it is able to slow or stop the growth of the tumour cells, this should allow us to develop an anti-cancer therapy that may complement or even replace existing treatments. We also aim to better understand the ways in which the protein has its effect and whether defects in the genes coding for this, and related proteins, may affect the nature and treatability of different kinds of medulloblastoma, which will be useful in diagnosis and treatment of the disease. An ‘in silico’ screen of a large medulloblastoma database has already shown some of these genes to be associated with particular disease subtypes.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>First, we will prove whether our naturally-occuring protein, or the pathway through which it acts, could be used to treat certain types of medulloblastoma. Second, we will show whether defects in the genes</td></tr>
</table>

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

animals could benefit from the project)?	coding for this protein, and/or other proteins in its pathway of action, are important modifiers of the disease, ie whether they may make the disease more or less aggressive. This would be of significant clinical benefit, providing important diagnostic and prognostic information.
What species and approximate numbers of animals do you expect to use over what period of time?	We will test whether our protein works on cells in vitro, which will require the sacrifice of a relatively small number of mice with tumours (around 30 per annum) to determine whether the protein is likely to work in vivo. In principle, the subsequent in vivo experiments should require about 50 animals for each condition to be meaningful, requiring around 300 animals per annum in total. However, we expect to considerably reduce this by looking, not simply at tumour incidence, but also at tumour size and its growth rate. We will also use less detrimental, yet diagnostic features at much earlier stages (3 weeks), which should allow us, not only to look at fewer animals (perhaps one third the number), but also to study animals in which 'full-blown' tumours have not yet developed. In all cases, animals will be humanely sacrificed at least as soon as the effects of the tumours are detected, but we expect the proposed refinements will allow us to do this well before the effects of the tumour becomes apparent.
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 effects in our experiments result from the breeding of mice engineered to develop medulloblastoma. If allowed to live for as long as one year, around 15% of these mice will develop the disease, which ultimately causes ataxia (inability to control movement) and death. Crossing these mice to mice carrying defects in genes coding for proteins thought to be involved in controlling the growth of medulloblastoma cells may in some cases accelerate the appearance of these symptoms. The level of severity is moderate if medulloblastoma develops, but the majority of our experiments will study animals at stages before this develops and the animals will simply be humanely culled and their tissues used for in vitro experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In principle, this study might be done with human medulloblastoma cell lines, but those that are available have accumulated many mutations in culture that make them resistant to drugs we know are effective against tumours in humans. This makes it likely useful drugs would be missed using these lines. Getting cells directly from human brain tumours is obviously very difficult. The mouse medulloblastoma model is very precise, exhibiting all of the symptoms associated with

	medulloblastoma in humans, providing an accurate test of the efficacy of our protein. Nonetheless, we will also use cell lines to try to reconstruct some of the important features of medulloblastoma so that much of our testing can be done in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	See notes on numbers above
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.	Our experiments contain a number of refinements that will reduce the suffering of the animals. First, the sophisticated use of genetic tools means that we can carefully control when the gene defects that give rise to medulloblastoma occur. In this way, much of the breeding that is required can be done with animals that show no abnormalities. Second, most of the testing for the efficacy of the protein will be done in culture with tissue derived from the medulloblastoma model animals, rather than in vivo. Third, we have identified early signs of the disease that we can monitor instead of waiting for full tumour development, which means that we can use fewer animals for shorter periods and still get meaningful results.

Developing an implantable vagus appetite regulator
Obesity, diabetes,

- Summarise your project (1-2 sentences)

We aim to develop an implantable electronic device to regulate vagal nerve function. This device will be developed to regulate appetite and to treat obesity in people.

- 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.

Obesity is a major source of mortality and morbidity. Currently it is estimated that at least one million people a year in Europe die as a result of obesity. There a currently no effective treatments for obesity except for bariatric surgery which is expensive and carries inherent risks. Therefore more effective treatments are urgently required. It is known that the vagus nerve has an important role in the regulation of appetite. Devices exist that stimulate the vagus nerve but they cannot be individualised and stimulate the entire nerve. We will develop a device which can learn the vagal inputs associated with satiety in individuals that can stimulate individual neuronal branches to prevent side effects. The nature of the work on a complex integrated system is such that animals are required.

- Outline the general project plan.

The initial stage of the project will be optimisation of the device. Each new design of device will be tested in rats and then ferrets. There will be a reiterative process of design test and further optimisation. At this stage all of the work will be conducted in animals that are maintained under general anaesthesia therefore preventing any suffering. During the studies the animals will be administered with hormones or nutrients which mimic satiety and stimulate activity of the vagus. This will allow the collection of the data necessary for the project with minimal suffering. Once we have reached a near final design we will test the design in pigs. The initial part of the study will be the same as that in previous sections with the animal maintained under general anaesthesia. This will allow us to optimise the final design of the implantable device. Once the final design has been reached the final stage of the project will be to test it long term in pigs. In this phase a device will be implanted into pigs under general anaesthetic using a keyhole approach if possible, to minimise suffering. We will then measure the food intake and body weight of the pigs to test the effect of our device on weight gain and obesity. The species of animals have been chosen as they are the best models of the physiology and anatomy of the human vagal nerve.

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.
For the most part the work will be conducted in animals maintained under general anaesthesia the entire time and all surgery will be performed under general anaesthesia. Because most of the project will be conducted in anaesthetised animals we do not expect it o result in any adverse effects. For the recovery surgery we expect the adverse effects to be minimal mainly pain associated with the surgery which will be controlled with analgesia. The other likely adverse is weight lose.

Predicted benefits:
Outline in a few sentences how science will advance, or people or animals will benefit from this project.
The project is to produce an implantable device that regulates appetite. If successful this will be developed as a treatment for obesity. Currently there is no widely applicable treatment for obesity, which currently kills one million people a year in Europe. An

effective therapy will therefore have enormous potential benefits in terms of human health and wellbeing.

- 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.

We expect that during the life time of the project the total number of animals required will be no more than 500 rats; 200 ferrets and 150 pigs.
The number of animals used will be minimised using a number of strategies. For the majority of the project studies will be of a paired design, where each animal acts as its own control. This approach greatly increases the statistical power of the studies and reduces the number of animals required. The other approach used will be to use an adaptive design for the studies in the early phase of the project. This means that the data will be analysed during the course of the experiments. Experiments will be stopped once sufficient data has been obtained to reach statistical significance.

- 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 project is designed to develop an implantable device which can monitor and stimulate vagus nerve activity to modulate food intake. It is not possible to achieve these aims without the use of animals. Some of the preparatory work has been performed using isolated frog sciatic nerves and where these provide a viable alternative they will be used in the ongoing project. However, isolated nerves preparations will not provide the detailed information required for this project. The isolated preparations are also incapable of providing the information regarding changes in response to physiological modifiers of energy homeostasis.

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

The initial work in the project will be conducted in rats. These have been chosen as they represent a reasonable model of the anatomy and function of the human vagus nerve and therefore allow the enabling technology to be tested in this system. Once we have established the technology in rats we will then carryout further testing in ferrets. These have been chosen for this stage of the work as they represent a model of vagus physiology and anatomy which more closely resembles that of humans than the rat model does. This will allow further development and validation of the chosen electrode architecture and functionality. The final phase of the project will use minipigs. These represent the best model for human physiology, anatomy and function available. This will allow us to finalise the architecture and design of the implantable electrode to be used. In this phase we will test the viability of this approach to regulating long term energy homeostasis. The long term study is being carried out in minipigs as these are the best model of human physiology and it is only at this stage that an implantable wireless device will have been developed. Before this stage a wired set up will be used, as this will be optimal for the development phase. Prior to the long term studies work on anaesthetised animals will be sufficient to allow the development of the prototype devices and the data required up to this point. This will minimise the suffering of the animals.
The use of minipigs for the long term studies also allows the potential for further refinement since it may be possible to undertake the operations using a laproscopic approach. Thus further minimising suffering.

Germ cell development and embryogenesis
Chromosomes, Trisomy, Infertility, Germ cells, Embryogenesis

- Summarise your project (1-2 sentences)

Chromosomal abnormalities such as trisomies concern infertility, spontaneous abortion and congenital diseases. In this project genetically modified animals are generated and bred in order for us to study chromosomal development and cell division during germ cell differentiation and embryogenesis.

- 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.

Chromosomal abnormality-related congenital diseases are common. For example, Down syndrome, which is caused by trisomy 21, is recognised more than 0.1% of live births. In addition, chromosomal abnormality is one of leading causes of infertility and spontaneous abortion. The origins of chromosomal abnormality are assigned to chromosomal errors that have happened during cell division in germ cell development, namely spermatogenesis in males or oogenesis in females, or early embryogenesis after fertilisation. However, how chromosomal errors happen or even more fundamentally how chromosomal development is correctly accomplished in healthy germ cells and embryos has been poorly understood. Other questions such as why incidences of trisomy and miscarriage increase along with maternal ageing have not yet been addressed. Our research aims to increase our understanding on physiological as well as pathological mechanisms for cell division and chromosomal development during germ cell differentiation and embryogenesis in mammals by genetically tractable approaches. In this project animals with genetic alterations will be generated and bred to supply tissues and cells required for our research programme.

- Outline the general project plan.

Genetically altered animals serve as valid animal models for human diseases. Studying their phenotypic abnormalities will clarify relationships between genetic causes and phenotypic consequences. In this project genetically altered animals are generated and bred. Tissue and cell samples will be taken from the animals after humane killing and their germ cell development and embryogenesis will be studied. To examine the effect of DNA damage to chromosomal development a small number of animals may be irradiated at low doses to induce recoverable levels of DNA damage to the cells. Tissue and cell samples will be taken from the irradiated animals after humane killing and their germ cell development and embryogenesis will be studied.

- 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.

We plan to generate genetically modified animals, which involve procedures including surgery that will be performed under appropriate anaesthesia. The procedures follow well established and widely accepted methodologies, it is unlikely that they result in more than temporary pain, suffering or distress to the animals.

We will breed genetically altered animals, which requires genotyping. Sampling of genotyping materials (small biopsies such as ear notches) from animals will be negligible and of mild severity at most.

Effects of genetic alterations for the majority of animals in this project will be negligible and of mild severity at most. As unpredictable phenotypes it is possible that genetic alterations might cause moderate severity suffering to minor fractions of animals. These effects are monitored and minimised by appropriate breeding, husbandry and/or veterinary measures.

A small numbers of animals may be irradiated at low dosages to induce recoverable levels of DNA damage. Since the doses of radiation will be more than 10 times lower than those used for cell depletion (e.g. for bone marrow transplantation), this should not lead to adverse health problems. Nevertheless animals will be preconditioned for irradiation and post-irradiation health monitoring will be conducted to minimise potential pain, suffering or distress.

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

Despite of importance, biology of germ cell development and embryogenesis has been poorly understood because of difficulties in experimental techniques and accessibility. Our research programme using animals provided by this project will make scientific advancement in understanding of chromosomal development and cell division during germ cell differentiation and embryogenesis. Outcomes of our research will benefit human health by improved diagnosis and treatment of chromosomal abnormalities.

- 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.

22,500 mice
We use the mouse, because it is the lowest mammalian species and the best mammalian species that allows genetically tractable experimental approaches. Genetically altered mice serve as vital animal models for human diseases. Studying their phenotypic abnormalities will clarify relationships between genetic causes and phenotypic consequences.

Animals used for each experiment are specified by sexes, genotypes and ages. Fractions of experimentally useful animals will be dependent on natures of genetic alterations. In the simplest knockout approach the chance to obtain knockout animals will be one-eighth in the Mendel's rule and sex preference. In more complex genetic alterations the chance will further decrease. Therefore the total animal number bred in this project will become larger then other projects that do not involve genetic approaches. We will plan animal breeding in the right scale and timing so that we do not breed excessive animals. The breeding plan will be directly managed or supervised by experienced scientists.

- 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 our research programme we will study mechanisms for chromosomal development and cell division during germ cell differentiation and early embryogenesis using genetically altered animal models. Since mammalian gametes (i.e. sperm and eggs) and their precursor germ cells (e.g. spermatocytes and oocytes) are only available from animals, our research requires animals.

We are aware that there have been reports in the past that described methodological development of in vitro gametogenesis. However most of such experiments have not been reproduced by research groups other than the authors. It is also possible that gametogenesis reported in such articles may be very different from the physiological one taking place in vivo. Therefore, usage of animals is essential for achieving our research aims.

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

We follow standard procedures for generation of genetically altered animals that has been widely accepted as procedures with the least suffering. For sampling of genotyping materials we will perform ear notching as the default technique that causes the least suffering.

Effects of genetic alterations for the majority of animals in this project are expected to be negligible and of mild severity at most. As unpredictable phenotypes it is possible that genetic alterations might cause moderate severity suffering to minor fractions of animals. These effects are monitored and minimised by appropriate breeding, husbandry and/or veterinary measures.

We expect the doses of irradiation used in this project will not lead to adverse health problems. However, we will minimise the stress and suffering by preconditioning and post-irradiation health monitoring, husbandry and/or veterinary measures.

Genetic control of early mammalian development
Mouse, embryo, transgenic, development

- Summarise your project (1-2 sentences)

  The project aims to identify how cells go from a naïve state of differentiation, where they have a very broad developmental potential to a fully differentiated state, where they have a very restricted developmental potential and perform the unique functions required by the tissue or organ they are in. We will identify the pathways and genes that direct the first steps of this differentiation process.

- 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.

  During embryonic development cells go from a naïve state to acquiring all the information that is necessary for them to perform the functions required in the different tissues and organs of the body. During this process an embryonic cell must go through a number of maturation steps before it can perform the complex tasks that are required of the terminally differentiated cell, for example a neuron. Each of these maturation steps involves a decision point where the cell decides between multiple terminal fates. As development proceeds, the range of fates available to a cell decreases and therefore its potential becomes limited.
  Very little is known about how the earliest cell fate decisions are taken and how a cell goes from having the potential to contribute to every terminally differentiated cell type in the body to having its potential restricted with every maturation step that occurs during development. We are interested in understanding how these decision between different fates are taken, what genes control them and in what way does each specific decision affect a cells potential. This information is essential for the safe and efficient use of pluripotent stem cells in regenerative medicine.

- Outline the general project plan.

To study the genetic control of early embryonic development we generate transgenic mouse lines that carry mutations in genes that are developmentally important. We analyse these mutations and compare them to normal development to understand what is the importance of specific gene pathways for the formation of the different tissues and organs.

- 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 vast majority of them will have the following experience – after being born and weaned, they will have a small tissue sample taken from the ear. Later, they may be set up to mate with a mouse of the opposite sex. Females will generally be killed a few days after they have been mated, so that embryos can be dissected out of them. Males will be retained for use in further crosses and then, before getting old and infirm, will be killed. A small proportion of the female mice will have surgery performed on them under general anaesthesia, in order to act as recipients for founder transgenic embryos and eventually, to act as foster mothers for these transgenic founder stock.

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

This project will identify the steps that take a cell down the differentiation pathway and identify the genes that play important roles during early development. It has become clear that genetic pathways responsible for orchestrating early embryonic development are remarkably conserved amongst vertebrate species. Although first active during embryogenesis many of these pathways are used again in the adult, especially during processes involving tissue renewal. Consequently, the genetic analysis of development in the mouse has direct relevance to the molecular basis of congenital abnormalities and other pathological conditions, such as neoplasia and defective tissue repair, in humans. Furthermore, the information harnessed in this project is essential to direct pluripotent stem cells in a safe and efficient manner into cell types that are important for regenerative medicine.

- 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.

Approximately 21,000 mice will be used over a five year period in this project. Mice are the ideal animal for this type of study for several reasons: mice have been studied for a long time by geneticists, so a large body of knowledge exists on which we can build; mice are very amenable to transgenic technology, which is used a lot in this project; mice are mammals and their development more closely resembles that of humans than other vertebrates like fish (which for instance, do not have a four chambered heart like humans and mice). We are very aware of the need to reduce the number of animals used. It should be emphasized that where possible we use in vitro models of development, such as embryonic stem cells. However, the in vitro differentiation of stem cells can only take us so far in our attempts to understand development as it cannot recapitulate the complex tissue interactions that occur in the embryo, and for this reason we always have to go back to the mouse embryos as our model system. Where available we will seek to import pre-existing transgenic or mutant lines rather than create new transgenics.

- 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.

Studies of this sort have to be performed with animals because we still do not understand enough about mammalian development to be able to model it using computers. Moreover, the specific tissue interactions and movements involved in this complex process cannot be accurately recapitulated in cell culture. In the lab we use as much as we can complementary systems such as embryonic stem (ES) cells to study how early embryonic development is controlled. However, the in vitro differentiation of ES cells can only take us so far in our attempts to understand development as it cannot recapitulate the complex tissue interactions that occur in the embryo, and for this reason we always have to go back to the mouse embryos as our model system.

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

Our work is related to human genetic conditions and to be adequate animal models the phenotypes are necessarily of moderate severity in some cases. However, the vast majority of animals generated or maintained will have minor or no phenotypes. Experiments involving surgical manipulations are performed only when there are no

alternatives, or when they allow a significant reduction in the numbers of animals that would otherwise have to be used to obtain similar data. In all cases, the numbers of surgical manipulations will be kept to a minimum compatible with valid statistical outcomes (seeking advice when necessary).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Novel Immunotherapeutic interventions for cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunotherapy, solid cancer, localising agents,</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>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>Our project aims to use novel minimally toxic localised immunotherapy treatments to inhibit all stages of cancer.

The overall aim of our project is to test the effectiveness of these novel immunotherapeutics against tumour growth in established murine models of cancer.

Within the overall aim, we have four objectives for the research
1. To test the effectiveness of these novel immunotherapies compared with the same immunotherapeutics in a non-localised form, on tumour progression, and anti-tumour immunity in mice.
2. To confirm that these agents are not detected in the blood of the animals (i.e. systemically).
3. To identify anti-cancer therapies (including vaccines) that can work in combination with the above agents to boost efficacy.
4. To study how solid cancers such as prostate cancer can weaken the immune system -so that we can also discover new targets that can be treated with immunotherapy</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>We believe that this project could identify effective low cost immunotherapies suitable for all stages of cancers that can potentially save millions of lives.</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	
What species and approximate numbers of animals do you expect to use over what period of time?	We propose the use of mice, which have immune systems that are similar to that of humans. We anticipate that 5000 animals will be used for the duration of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be injected with tumour cells so that tumours grow either as a mass or within the lungs. Animals will be monitored frequently to ensure that they are not in pain or distress and we will use an established acceptable end point for experiments based on a maximal tumour size, and not death of animals. We anticipate very few adverse effects but, if these are seen, treatment will be ceased and/or animals will be culled humanly
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To achieve the objectives of the project, it is vital to use animal models: The tumour environment is complex and agents that activate immune cells to kill tumour cells in-vitro may not work in humans. Although slight differences occur between murine and human immune systems, mice challenged with tumour cells are the most appropriate model for screening of preclinical efficacy of our drugs. Many of our reagents, such as cytokines have an equivalent effect on murine and human immune cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce animal numbers in 3 ways: **1)** We will only use agents in-vivo that are confirmed as being active in-vitro for boosting tumour specific immunity This will reduce animal numbers at least 5-fold as some of our preparations will be inactive in-vitro 2) Our experiments have been designed with the help of a statistician to use the lowest number of animals which will give statistically valid results. 3) We will have detailed write ups and record keeping for all experiments noting all observations on animal health and welfare.
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.	**Choice of Models**: The murine tumour challenge models we are using have been established for many years as the simplest way of assessing primary and metastatic tumour progression, with minimal suffering to the animals and obtaining maximal amounts of data. **Minimizing Suffering** We will undertake a number of refinement

	measures for our experiments to minimize animal suffering and therefore minimise variations in our measurements. 1) For injections of cells or other therapeutics, we will use needle sizes with the maximal gauge possible for delivery of the agent/s so that the animal suffers the least amount of pain. 2) For injections, the area to be injected will be treated with ELMA cream to minimise discomfort during and after injection. 3) Body condition will be monitored as a whole using a number of criteria such as weight loss (or weight gain combined with thinning of the animals), condition of fur, lack of activity, signs of respiratory distress and alertness, compared to non-tumour-induced control animals in the same experiment.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Orofacial development, homeostasis and repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Teeth, tissue repair, regeneration, salivary glands</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)[9]</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>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</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>To study how teeth and salivary glands develop in the embryo and grow in adults. To understand how cells can naturally repair damaged teeth and salivary glands and to use cells to generate new teeth and salivary glands for transplantation.</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 enhancement of natural repair mechanisms and an understanding of the genetic pathways that can cause tooth and salivary gland abnormalities. The development of cell-based methods to regenerate teeth and salivary glands following loss of disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>No more than 41 000 mice over a 5 year duration</td></tr>
<tr><td>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?</td><td>We expect minimal adverse effects as the vast majority (80%) of the animals will have minimal (mild) discomfort (associated to breeding). Any animals which undergo an operative procedure will have adequate peri-operative anaesthesia to minimize discomfort and all procedures will be performed in aseptic conditions to minimize the risk of infection. The terminal endpoint of all animals will be humane euthanization</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot</td><td>This research is focussed on investigating organs that are not present in non-animal species.</td></tr>
</table>

9 Delete Yes or No as appropriate.

10 At least one additional purpose must be selected with this option.

use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible experiments on teeth and salivary glands will be combined in the same animal. In addition the culture of organs will also be used to answer some specific questions where one mouse can provide tissue for several cultures.
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 are the animal of choice for genetic-based studies of development. The ultimate aim of this research is to understand the control of human tooth and salivary gland development, growth and repair and thus a mammalian model system is needed. The surgical procedures used have all been developed and refined in the mouse as have the growth ex vivo of organs.