Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 9

Projects with a primary purpose of: Translational and Applied Research - Non-regulatory Toxicology and Ecotoxicology

Project Titles and key words

1. **Genetic Toxicology (Safety Assessment)**
    - Genetic, toxicology, safety assessment, rodents

2. **Chemical Agent Studies and Medical Countermeasures**
    - Chemical agent, Medical Countermeasures

3. **Evaluation of pharmacokinetic properties of novel drug candidates**
    - Pharmacokinetics, novel drug candidate, mouse, rat, *in vivo* studies

4. **ADME and Pharmacokinetic Studies**
    - Metabolism, ADME, Pharmacokinetics, Animal Health, Environmental Fate

5. **Biocompatibility of medical devices and materials**
    - Biocompatibility, irritation, sensitisation, systemic toxicity

6. **Can we predict chemical mixture toxicity?**
    - Fish, chemicals, mixtures, modelling, toxicology

<table>
<tr><td>PROJECT 1</td><td>Genetic Toxicology (Safety Assessment)</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetic, toxicology, safety assessment, rodents</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>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</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>New medicines are required to treat a range of human diseases, many of which currently have no effective treatment. The main objective of this project is to assess the potential of substances in pharmaceutical development to cause genetic damage. This genotoxicity is assessed in animals so that clinical trials of new medicines in healthy volunteers and patients can be performed more safely.

Regulatory guidance documents from the European Agency for Evaluation of Medicinal Products and the United States Food and Drugs Administration describe the types of animal tests to be conducted in development of new medicines, according to the anticipated clinical use and target human population.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science</td><td>The overall benefit of this project is that it supports the development of safe, new medicines to improve</td></tr>
</table>

could be advanced or humans or animals could benefit from the project)?	the health and quality of life of patients by generating high quality data that is acceptable to regulatory authorities and enables company decision making. Output of work under this licence will enable future medicines to progress with reduced genotoxic risk and conversely will also help to stop the development of unsuitable compounds at an early stage, thus saving animals and resources. Without these studies progression of potential new medicines to early human studies and to patients could not occur. Data from studies will also be used to alert regulatory authorities to any potential safety concerns for substances already being administered to patients (e.g. specific breakdown products (metabolites) identified in human trials). In addition, studies will address issues arising during the production, manufacture and marketing of substances, (e.g. changes in production processes/locations, or drug impurity specifications) to permit a more efficient process to be implemented or a new batch of medicine to be used clinically.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year lifetime of this project: Rats 1800 Mice 1100
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?	Typical studies last 1-3 or 28 days and comprise daily dosing with the putative medicine combined with occasional blood sampling. At the end of the treatment period, blood and tissue samples are taken from terminally anaesthetised, or euthanased, rodents for analysis of chromosome damage/loss and/or gene mutation in the red blood cell population and/or DNA strand breaks in the liver. Regulatory authorities generally require characterisation of genetic toxicity at the maximum tolerated dose (MTD) to ensure robust evaluation of safety before administration to humans. The MTD is defined as the dose producing adverse effects such

	that higher doses, using the same dosing regimen, would be expected to produce lethality. It is therefore necessary to administer doses of the potential medicine that produce systemic toxicity, e.g. body weight loss, inappetence and transient lethargy. Initially, small dose range finding studies are used to establish a suitable high dose to be used in a larger definitive assessment, and because these will be the first animal studies that explore the high dose range, more severe adverse effects are anticipated in animals that receive high doses in these studies e.g. abnormal changes in posture, reduced activity and abnormal breathing patterns that do not show signs of recovery. All studies will be closely managed so the minimum number of animals experience significant pain and distress. Animals will be observed regularly to monitor changes in appearance and behaviour and action will be taken to alleviate any pain and distress, such as withdrawal of the animal from further dosing, reducing the dose (if appropriate) or euthanasia. Rarely, cannulae will be implanted under appropriate anaesthesia and pain relief *e.g.* for intravenous administration of substances or withdrawal of blood (e.g. to measure the absorption and distribution of the potential new medicine). This removes the need for repeated needle sticks in studies that require multiple intravenous doses or samples. Rarely, animals that have only experienced minor procedures, e.g. single blood sample, will be released from the authority of this licence for potential re-use after the Named Veterinary Surgeon has advised that the animals' general state of health and well-being has been fully restored.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Whilst alternatives to in vivo animal models and methods of screening in animals of lower species are being developed, there are currently no reliable models that can completely replace genetic toxicity

use non-animal alternatives	testing in animals; they remain a regulatory authority expectation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Continuous improvements in computer prediction models for genotoxicity and use of cell culture based assays (not involving the use of animals) do result in the majority of genotoxic chemicals being eliminated early from the medicine development process. This minimises the number of animals required for mandatory testing. The number of animals analysed is determined by current regulatory recommendations and/or expert working groups. Statistical analysis has been conducted, and where appropriate, the group sizes have been reduced to minimise the number of animals used and yet maintain compliance with the regulatory guidance and scientific validity. Following approval of the updated international guidelines for genotoxicity testing in Nov 2011, the recommendation to integrate *in vivo* genotoxicity assessment with general toxicology studies has resulted in a significant reduction in the number of animals used for standalone genotoxicity studies. Furthermore, when stand alone *in vivo* genetic toxicology studies are still required, refinements in the protocol to combine multiple endpoints into a single study will further contribute to the overall reduction in animal usage.
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.	Regulatory guidelines state that genetic toxicology studies in support of administration to humans should be conducted in one rodent species. Typically rats will be used because of the existence of large historical databases which help to assess the potential risk to humans. Prior to commencement of studies, experimental procedures will be reviewed by highly trained scientists and animal welfare specialists. Initial dose range finding studies with small numbers of animals will be used to assist in suitable dose selection for subsequent definitive studies using larger group sizes. Humane endpoints will be

	applied to limit the pain and distress experienced by the animals to the minimum required to achieve the objective(s) of the study.

PROJECT 2	**Chemical Agent Studies and Medical Countermeasures**	
Key Words (max. 5 words)	Chemical agent, Medical Countermeasures	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This work programme will develop therapeutic drug treatments and treatment regimens, against toxic chemical agents.	
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)?	Chemical agents may be used against the Armed Forces or against the general public by terrorists. Some of these agents interfere with the nervous system and disrupt bodily functions such as breathing and muscular function resulting in incapacitation and death. In order to protect against these effects, there is a requirement to develop and assess improved Up to 260 marmosets over a 5 year period.	
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 260 marmosets over a 5 year period.	

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 are likely to experience signs of poisoning associated with chemical agent exposure. The severity of these signs will depend upon the amount of chemical agent administered and also the effectiveness of the MedCMs being tested. Signs can range from; constriction of pupils, tongue protrusion and salivation to tremor, convulsions and death. Animals will either die or be culled at either a humane endpoint or the end of the study. There may be some brief discomfort (bruising) following the withdrawal of blood samples or administration of injections. There is potential for some medical countermeasures to cause short term moderate effects. For some experiments it may be required for the animals to be surgically implanted with telemetry devices (which will be used to monitor physiological parameters) or with drug delivery devices. This will lead to some transient discomfort but pain killers and antibiotics will be administered. Surgical procedures are always carried out under either general (telemetry or drug delivery devices) or local (temperature transponders) anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unlike other areas of pharmaceutical development, few opportunities exist to demonstrate the effectiveness of MedCMs to chemical agents in humans. Therefore there is a greater requirement for the extrapolation of animal-derived data to man. The physiological effects of chemical agent poisoning are complex, involving a number of organs and systems, and cannot be replicated invitro. Therefore, there remains a requirement for whole animals to study the interactions of these systems. By using marmosets it is possible to assess indices such as cognition, behaviour, motivation and incapacitation which provides a more detailed picture of the effectiveness, or otherwise, of the MedCMs

	being tested. This could not be achieved in an in-vitro study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Only MedCMs that have been demonstrated to be efficacious in the guinea pig model will be transitioned to the marmoset. The guinea pig is a long established model of chemical agent poisoning and is a better model for man than other small animal species. Consequently MedCMs that have shown efficacy in the guinea pig should be efficacious in the marmoset. The statistical advice will be provided on the design and interpretation for each study. For inhalational exposures, by using online realtime calculations of chemical agent vapour concentration it can be ensured that an accurate exposure is delivered which will also minimise the number of animals used.
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.	Whenever possible humane endpoints will be determined. In our previous experiences working in this field, death from volatile chemical agent poisoning typically occurs very rapidly with time limited suffering. The marmoset allows us to assess and understand the level of protection provided by MedCM against chemical agent poisoning and particularly against incapacitation in terms of clinical signs, behaviour and cognition. These parameters can all be measured in individual animals thereby keeping the number of animals used to a minimum.

PROJECT 3	**Evaluation of pharmacokinetic properties of novel drug candidates**	
Key Words (max. 5 words)	Pharmacokinetics, novel drug candidate, mouse, rat, *in vivo* studies	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to evaluate the pharmacokinetic (PK) properties (absorption, distribution, metabolism and excretion) of novel compounds in mice and rats and assess whether they support further development of the compounds as potential drugs, targeting areas of unmet clinical need. This *in vivo* evaluation of drug candidates is an essential part of the drug discovery process. *In vitro* data is utilised in the selection of compounds for animal testing, however, no *in vitro* techniques can fully predict the *in vivo* behaviour of all compounds. By obtaining appropriate *in vivo* PK data early in the programme of work, the most suitable compounds can be selected for progression into pre-clinical and then clinical efficacy studies, with a much greater chance of success. The majority of studies involve the administration of a single low dose of the compound by a selected route and taking timed blood samples for the assessment	

	of compound levels. Standard PK parameters will be determined from these data (e.g. half life, apparent volume of distribution, clearance). Results from studies carried out under this licence can be used to optimise dosing regimes prior to testing suitable compounds in animal models of disease. Data produced can also be used to improve the design of future compounds.
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)?	The long-term benefit of this project will be the selection of novel compounds for drug development with a higher probability of success in the clinic, so improving the availability of new medicines in areas of unmet clinical need. In the short term evaluation of PK properties of compounds will filter out those with unfavourable characteristics so that only those most likely to be efficacious will be tested in future animal models of disease. The data generated will enable clear decisions to be made and will be used to improve the design of future compounds.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice are requested on this licence. It is anticipated that no more than nine hundred rats and one thousand seven hundred and fifty mice will be required over the course of this 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?	All staff involved in conducting proposed studies are highly experienced, so adverse effects from routine procedures such as dosing (by various routes of administration), sampling and use of anaesthesia, are infrequent. Compounds will be selected on the basis of determined *in vitro* properties. Doses will be kept as low as possible and all animals on study will be closely monitored. The severity level for Protocol 1 is moderate and Protocol 2 is Non-recovery. All animals will be humanely killed at the end of all protocols.

<table>
<tr><td>PROJECT 4</td><td colspan="3">ADME and Pharmacokinetic Studies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Metabolism, ADME, Pharmacokinetics, Animal Health, Environmental Fate</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">Five</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>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</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">In the development of new animal health and human drug therapies there are many challenges that must be overcome by a potential new medicine before it is considered to be safe for administration. The main hurdles are to get the material into the body in just the right amount, at the right time and for long enough. The work in this project will check to see whether such new materials are able to get into the blood or correct part of the body in sufficient amounts to have the required effect and then to check how the body might change the material itself before it is excreted from the body.

In order to investigate how the body takes up and then handles potential new treatments, we particularly investigate the following key aspects: - absorption, distribution, metabolism and excretion.</td></tr>
<tr><td>What are the potential benefits</td><td colspan="3">Metabolism and pharmacokinetic studies are major</td></tr>
</table>

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	determinants of evaluation of potential new therapies and as such these studies: • permit optimal selection of potential medicines (both for humans and animals) for progress to clinical trials • aid optimisation of the formulation, dose and route of administration used in safety assessment and in clinical use • play an integral part in the safety and clinical assessment of a new treatment • help in the development, publication and dissemination of refined procedures • facilitate the entry of valuable new therapies onto the market
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of studies in animals will involve administration of potential therapies to mice (about 5,000 used per year) and rats (about 1,000 per year) followed by blood samples being taken at various times after dosing. Studies are carefully designed so that any side-effects are minimised by using all the information known about the dose material. A much smaller number of experiments may be carried out in dogs, pigs, goats, sheep and hens (less than 50 per species per year) - the study designs being similar to those used for the rat.
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 expected adverse effects in all our studies are expected to be minimal and the expected level of severity to be mild. At the end of a study animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We conduct many research projects involving a wide variety of methods that do not require the use of animals including use of cell culture and studies using ethically-sourced donated human tissue. Nevertheless despite advances in non-animal methods it is still required to use animals where there are no viable alternatives in order to enable additional much-needed advances in medical

	knowledge and development of new treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We only ever use the minimum numbers of animals required to establish variation of response and as laid down specifically by international regulatory guidelines.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species we use are the most refined with regard to achieving study objectives and over 95% of animals used involve rats and mice. Our animal facilities are staffed by dedicated animal scientists who are responsible for the care and welfare of the laboratory animals and their environments. We actively support the 3Rs and are innovative in developing techniques to minimise potential harm and in enhancing animal welfare.

<table>
<tr><td>PROJECT 5</td><td>Biocompatibility of medical devices and materials</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Biocompatibility, irritation, sensitisation, systemic toxicity</td></tr>
<tr><td>Expected duration of the project (yrs.)</td><td>5-years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>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</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>The overall aim of this programme of work is to evaluate the safety and biocompatibility of novel materials and devices that will come into contact with body tissues before, during or after a surgical procedure</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>To assess the safety of materials or devices intended for human use to reduce or eliminate any adverse effects.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over 5-years
Rabbit – 1000
Guinea pig – 1000</td></tr>
</table>

	Rat – 2000 Mouse - 2000
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 nature of the tests will involve skin irritation/ sensitisation and acute/chronic systemic toxicity of no more than moderate severity. Animals will be killed at the end of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Laboratory *(In vitro)* testing cannot fully replicate the in-body (*in-vivo)* physiological conditions required to test the safety of novel devices, therefore animal tests are necessary.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required for each test is either clearly defined in established methods detailed in international standards or will be determined to provide a meaningful outcome.
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.	Animal suffering will be minimised by proactively refining the materials tested by: (a) consultation with people with expertise in toxicology, animal welfare and statistics, (b) Laboratory (*in-vitro)* tests to refine the biomaterials and hence, reduce the level of pain, distress or lasting harm experienced by the animals. The species identified and the surgical techniques used are clearly defined in established methods detailed in international standards

PROJECT 6

Can we predict chemical mixture toxicity?

KEYWORDS

fish, chemicals, mixtures, modelling, toxicology

Purpose

Protection of the natural environment in the interests of the health or welfare of humans or animals

Summarise your project (1-2 sentences)

The ultimate aim of this work is to develop a computer model that will predict the effects of complex mixtures of chemical pollutants on wild fish. This project will determine the sub-lethal effects on fish growth, reproduction and behaviour from chronic exposure to representative chemicals, both singly and in mixtures, providing data to validate the computer model.

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.

Validated models predicting the effects of complex chemical mixtures of anthropogenic chemicals are required for chemical risk assessments to ensure wildlife and the environment are protected. Developing computer models to predict toxicity (based on data from molecular and physiological responses) will address the 3Rs by replacing and/or reducing the need for testing chemicals on animals.

Outline the general project plan.

The maximum total number of 3-spined sticklebacks (laboratory bred) used will be 820, during a single chronic study over a 4-month period. We have significant expertise in working with 3-spined sticklebacks which are well suited to laboratory culture. Our in-house strain will have consistent physiological responses, is of known provenance, and free from pathogens. No acute toxic effects are expected because the concentration at which each chemical will be used is at least 3 orders of magnitude lower than lethal dose, and similar to those reported in the environment. The anticipated adverse effects are restricted to MILD, i.e. disturbances of physiology (e.g. reduced growth, reduced reproductive output). All animals will be humanely euthanized at the end of the study. Although no toxic effects are anticipated, unexpected adverse effects may manifest during the exposure period. If visible signs of ill-health or stress in an individual are observed, the animal will be humanely killed and the frequency of monitoring will be increased. If 2 or more fish in a tank display signs of ill-health or stress, that particular treatment will cease and the

animals euthanized.

• **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.**
Most of the procedures employed in this project do not cause more than transient discomfort to the animals or lasting harm.

Usually the genetically modified mice we generate exhibit only mild changes in physiology. However, it is possible that muscle growth (and therefore whole body) growth may be more severely compromised, for example by up to 50%. This results in weaker animals, which need additional care and are culled if they show signs of discomfort.

A very important aspect of this project is the assessment of the ability and efficiency of muscle regeneration (muscre must regenerate throughout life to maintain tissue mass and this ability decreases greatly with age). Regeneration is modelled by inducing a modest (and reversible) muscle injury, using well-established protocols. This is either snake venom injection (breaks down muscle fibres but does not affect satellite cells which regrow the muscle) or a freeze for a few seconds (kills all myofibres and cells, and so tests the ability of satellite cells to travel from neighbouring fibres and repair).

Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
This project will advance our understanding of muscle stem cell (and therefore other tissue stem cell) function in a variety of physiological conditions. Muscle frailty is a major problem in the elderly, leading to falls, lack of confidence, isolation and possibly depression. The research within this project should lead to ideas for the development of strategies to reduce diminished muscle function during old age.

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 estimate we will use up to 3,000 mice per year during this study. Mice are used because they provide a good model for many aspects of human muscle function, they provide sufficient material for analysis, and because advanced genetic models already exist (thus avoiding needless repetition). One reason that we use this number of mice is that some complex breeding crosses only generate a small number of mice carrying a revealing gene combination. However we do try to ensure as much efficiency as possible (e.g. production of ‘control’ and ‘test’ animals within

each litter).

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

We can't replace the fish by cells or other non-animal methods because evidence is needed on animal responses to chemical mixtures to parameterise and validate the non-animal computer model. The number of fish in an experiment is a product of the number of treatments, the number of replicate tanks per treatment, and the number of fish per tank. A high number of treatments (40 chemical mixtures combinations tested) is required to ensure that a robust computer model will be derived. The number of replicate tanks per treatment (2) is minimised and is based upon prior demonstration of consistency between replicates. The number of fish per replicate (10 fish per tank) is considered the minimum to allow normal social behaviour and gain a representative sample of a population.