Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 32

Projects with a primary purpose of: Translational and Applied Research – Non-regulatory toxicology and ecotoxicology

Project Titles and keywords

1. **Zebrafish: an alternative model for drug safety & efficacy**
    - Epilepsy; toxicology; diabetes; pharmacology

2. **Investigative & Enabling Safety Assessment Studies**
    - Safety, toxicity, drug discovery/development

3. **Assessing the hazards of nanomaterials**
    - Nanomaterial, toxicity, safety, mechanism, inflammation

4. **Regulatory Aquatic Ecotoxicology Testing**
    - Aquatic, ecotoxicology, fish, freshwater

Project 1	**Zebrafish: an alternative model for drug safety & efficacy**	
Key Words (max. 5 words)	Epilepsy; toxicology; diabetes; pharmacology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Zebrafish are a credible biomedical model due to their close physiological and genetic similarity with humans. In addition, their small size (<5mm) means larval zebrafish can be used to test for drug side effects or effectiveness earlier in drug development than rats, mice and dogs. Early detection of side effects means fewer drugs will fail during development, which is financially and ethically (in terms of animals used) very costly. Consequently, this project has two main aims: develop and use zebrafish-based tests for human drug side effects; and develop and use zebrafish-based models of human disease for drug efficacy testing. Side effects to be investigated initially are seizures, developmental toxicity, renal, cardiovascular, auditory and visual impairment. Disease models to be investigated are epilepsy, diabetes, kidney failure and hearing impairment.	
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)?	Ultimately, this project will help improve the quality and effectiveness of new drugs. The side effects we are studying are major causes of drug failure during development. For example, kidney toxicity accounts for 20% of organ toxicity in animal studies. In addition, diabetes, epilepsy, kidney failure and hearing impairment are globally significant human	

	health issues: epilepsy occurs in 1-2% of the global population, of which up to 30% of people do not respond to current treatments. Our project will provide appropriate models in which to further our knowledge of these human diseases, and will help to develop better medicines that reach patients more quickly.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will use approximately 30000 zebrafish per annum, although 99% of these will be embryolarval (<14 days old).
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?	Five protocols are proposed: **1)** Generation of zebrafish genetic models involves alteration of genetic characteristics to allow the study of specific human diseases and their treatment. For example we can create animals in which particular organs or cells are more easily visible under the microscope; or other models in which we can induce a disease state to allow the testing of potential treatments. In the former case, the animals do not show any adverse effects associated with the introduction of the marker. In the latter case there may be adverse effects associated with the disease generated (e.g. seizures), but models can be designed so that they only occur in embryolarval offspring (and not adults) which are humanely killed at a very early age. **2)** Temporary genetic manipulation involves injection of substances into eggs that temporarily interfere with genetic processes. These allow us to test whether particular genes are involved in certain diseases thus allowing us to design drugs to target these pathways. This approach is only undertaken in very young fish which are humanely killed before maturing. **3)** The maintenance and breeding of genetically modified fish conveys no harm on the animals and by the time animals are of a breeding age, any harmful features have been removed from the stock pool. At the end of the optimal breeding period (usually 2 years), animals will be humanely killed. In some cases animals may be supplied to other establishments for use saving the generation of an equivalent model elsewhere, and minimising associated animal usage. **4)** Drug exposures for testing toxicity are usually by

	immersion after which animals are assessed for the signs of toxicity. For this we use a series of tests that have been shown to be representative of what is likely to happen in mammals including humans. The aim for this work is to undertake early side effect testing in a lower vertebrate (embryolarval fish) rather than in a rat, mouse or dog. At present we test for side effects such as seizures, developmental abnormalities, hearing and sight impairment and cardiovascular and kidney damage. Following exposure and assessment, all animals are humanely killed (usually <14 days old). **5)** Drug exposures for testing the effectiveness of new medicines are undertaken in animals in which we have already created a 'disease' to treat (**1 and 2 above**). Once this is done, we can test whether new drugs are effective in treating this disease, usually by immersion of the fish in water containing the drug. At present we are able to recreate models of epilepsy, diabetes, kidney failure and hearing impairment. The former is achieved through treatment with drugs and the latter three by selectively removing cells that infer normal organ function. At the end of the procedure, animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whole animal preparations are ultimately necessary to accurately recreate integrated organ system responses. In mitigation, the overall goal is reducing mammalian testing on drugs destined to fail. In addition, computer modelling of properties governing uptake and effects will be undertaken, thus potentially replacing the use of some animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every aspect of experimental design is geared towards reducing animal use: we routinely use statistics to determine the minimum number of animals needed; often use shared control groups; undertake pilot studies on fewer animals to ensure appropriate dose ranges; maximise information gained per animal used; and strive to combine endpoint measurements in the same animals wherever possible. The overall purpose is to reduce the number of tests undertaken unnecessarily on mammals with drugs that will eventually fail due to a poor side effect profile or low efficacy.

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 main focus of our work is to use (predominantly larval) zebrafish as surrogate models for mammals and ultimately humans. For safety studies, most procedures merely involve observation after drug treatment, and are conducted in very young animals (<10 days old). Consequently, adverse effects are most likely associated with unpredictable drug properties. To minimise suffering we continually review dosing levels as more information is gathered; share data between assays and operators; and frequently monitor animals and humanely kill them at the minimum level of suffering that meets the aims of the test. For disease modelling, adverse effects are most likely associated with the characteristics of the disease being modelled. Control measures include: using existing models to avoid the animal cost of developing our own; minimising unexpected adverse effects by using information from previously generated, similar, disease models; breeding out undesirable effects; and humanely killing animals at a point appropriate for achieving the aims of the experiment.

Project 2	**Investigative & Enabling Safety Assessment Studies**	
Key Words (max. 5 words)	Safety, toxicity, drug discovery/development	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	✓	Translational and applied research
		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)	To generate data to support the development of safe and effective novel medicines which therefore have the greatest benefit to patients in treating disease where there is currently a clinical unmet need e.g., cancer and diabetes.	
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)?	Contribute invaluable scientific data to support and progress the development of new medicines where there is a clinical unmet need. Enable the development of new medicines with the least chance of causing adverse effects and therefore be of maximum benefit to patients. For example a patient could receive the maximum amount of an anti-cancer agent needed to give maximum effect with little or no side effects.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats 9250 and Mice 3300 over 5 years A typical study will use relatively small numbers of animals (<50). It is anticipated that up to 3000 animals may be used per year (combined rats & mice).	
In the context of what you propose to do to the animals, what are the expected adverse	Studies will be conducted by dosing routes similar to those used in man, e.g. by mouth (orally) or by injection. The animals are then observed regularly	

effects and the likely/expected level of severity? What will happen to the animals at the end?	to monitor changes in appearance and behaviour. Procedures carried out during these studies include: a) Weighing: as a loss in body weight is often an early sign of harmful effects in animals b) Blood sampling or collection of urine for measurement of different components as changes in these may serve as early indicators of toxicity. Doctors for similar reasons often take blood and urine samples from humans. c) Electrocardiography (ECG) monitoring to assess changes in heart function (e.g. number of heart beats per minute). This technique is also used by doctors to assess heart function in humans. d) Animals may undergo surgical procedures for examples to implant telemetry devices for non-invasive longer term cardiovascular monitoring, surgical implantation of vascular cannulae, or surgical procedures undertaken in animals for non-recovery investigations. A degree of restraint or confinement may be required for some of the various dosing, sampling or assessment procedures. At the end of the study the animals are humanely killed by an overdose of anaesthetic. Where appropriate animals may be re-used for subsequent investigations. Samples of various organs are taken and examined under a microscope to ascertain whether the potential new medicine has caused changes that would prevent administration to humans. From experience, the majority of animals are expected to have mild adverse effects such as slight weight loss. A small percentage of animals may show more significant adverse effects e.g. more marked weight loss, or changes in appearance (e.g. ruffled fur in rodents) or behaviour (e.g. reduced activity) indicative of moderate severity. Humane end-points are applied, under veterinary guidance as necessary.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Prior to studies within this programme of work a number of in vitro (in cells) and/or computer program simulated tests will have been used to establish

use non-animal alternatives	early safety liabilities and risk associated with a target or novel agent. Only by using animals as part of a hypothesis driven experimental design can we validate the impact of these in vitro/simulated findings on disordered physiological processes and pharmacology, and ultimately the potential for adverse effects in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be set after consultation with a professional statistician. Animal numbers are kept to a minimum commensurate with meeting the objective of each study, and the endpoints being measured.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice have a well characterised pathophysiology, and are well validated for their use to measure safety/toxicity effects. The rat is generally the species model of choice, since there is a wide knowledge of the response of rats to various chemical entities and a wealth of knowledge and published information. Rats are big enough to provide repeated blood samples, thus requiring significantly fewer rats than mice to achieve the same objective. Blood volume taken for measures will be minimised by using micro sampling techniques which significantly reduce the volume of blood removed from each animal, but still produce high quality data. Best practice, for example the use of analgesics after surgical procedures will be employed to minimise suffering. Adverse effects will be kept to the minimum to achieve the objectives. Animals will generally be housed in groups and provided with specific materials to provide enrichment.

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<tr><td>Project 3</td><td>Assessing the hazards of nanomaterials</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Nanomaterial, toxicity, safety, mechanism, inflammation</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)</td><td>
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<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>
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<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>
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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>Technology has developed to make particles in very small sizes. The dimensions of these particles are in the nanoscale, (less than 100nm in size), which means they are thousands of times smaller than the diameter of a single hair. These new particles, known as nanomaterials, are made from many different substances (e.g. gold, silver, zinc oxide and titanium oxide) and are interesting because materials change their properties when their size is so small. The availability of a wide array of nanomaterials with exploitable properties has led to the development of many new and improved products including medicines, disease diagnostics, clothing, food additives, food packaging, antimicrobial coatings, water purification, paints, electronics, cosmetics and sports equipment. While the properties of nanomaterials offer many benefits to society and the environment, their widespread use and their unpredictable properties also present potential risks. Gaining information about the risks posed by nanomaterials to human health and the environment is essential for industry in order to understand how to manage and use their products,</td></tr>
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	for scientists when designing new nanomaterials and for regulators when trying to protect consumers and the environment. The toxicology research conducted so far for nanomaterials is largely limited to short term studies, often using cell cultures rather than humans or animal models. Currently, insufficient information is available to confirm whether many of the cell studies performed are able to predict responses that occur in the body, and so it is difficult to assess the toxicity sufficiently accurately using these techniques. Research using animals has demonstrated that nanomaterials can move around the body and accumulate in many places, but mainly in the liver. Our own research demonstrates, that the liver can respond in a variety of ways that might be associated with disease. However, the type of response generated by the liver appears to be influenced by the route of entry of nanomaterials into the body. Our data indicates that the liver response to injected particles can be predicted well by simple liver cell cultures. In contrast, after exposure via the gut or the lungs the liver response can be modified, reducing the ability of the cell culture system to predict the liver response. Much work is therefore required to better understand the toxicity of nanomaterials at the point of entry into the body (e.g. gut and lungs) or at distal targets (e.g. liver), to identify good indicators of toxicity, to better understand the long term effects of nanomaterials, to better understand the relationship between physical and chemical characteristics of nanomaterials and their ability to induce toxicity, as well as to develop more robust cell based alternatives to animal testing of nanomaterials. The proposed research aims to contribute to all of these areas. Specifically this includes development of alternatives to animal testing (in cells and computer models), assessing mechanism of toxicity, assessing how route of exposure influences toxicity, assessing how coating of particles with biological substances alters their toxicity and finally the long term effects of particles entering the body via the lungs and gut.
What are the potential benefits likely to derive from this	The results gained will contribute to an increased understanding of the hazards of nanomaterials that

project (how science could be advanced or humans or animals could benefit from the project)?	will be used to assess their risks, to develop protocols for hazard and risk assessment and for the development of guidelines or even legislation related to nanomaterial safety. The knowledge gained in relation to nanomaterial behaviour in the body will also be exploited in the development of new treatments for disease such as cancer and atherosclerosis by allowing improved targeting of therapies while minimising side effects.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (up to 1500) and mice (up to 1500) will be used for this study.
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 majority of studies to be conducted are anticipated to generate mild symptoms. On the whole, real life exposures are not anticipated in humans to be large enough to generate severe or lethal symptoms. In fact, it is difficult to publish data with large nanomaterial concentrations that would induce severe symptoms, due to the lack of relevance of such work. Several nanomaterials have now been tested in animal models, thereby providing information to inform the choice of dose(s) appropriate for future studies. When a new nanomaterial which is previously untested is studied for the first time, *in vitro* tests will be used to assess its cytotoxicity and ability to induce pro-inflammatory responses compared to other nanomaterials. The results of this work will be used to inform the choice of dose to be used *in vivo*. Where symptoms due exist they are more likely to occur following longer term exposures. Following exposure to nanomaterials all animals will be monitored closely for immediate symptoms and for those that develop over time. All animals are sacrificed according to Home Office regulations at the end of each experiment. This is essential in order to obtain tissues to assess the impact and localisation of the nanomaterials.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Where ever possible we avoid animal experimentation by using alternatives models and data. The objectives of this project licence include the

use non-animal alternatives	development of cell based alternatives to animal models. Data from animal studies will be required to assess the validity of the models, where possible this will be achieved through the use of historical data derived from animal studies, but for some additional studies will be required. For assessing the potential risks of nanomaterials, information from long term toxicity studies is required. No *in vitro* models currently allow such studies, however animal models with a relatively short duration (33 days instead of life time) have been developed for inhalation studies, thereby reducing the severity to the study. This inhalation model will be employed but also adapted for ingestion exposure, thereby reducing the severity of long term ingestion studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When animal studies are deemed necessary (due to a lack of good alternatives), the majority of work will be conducted in collaboration with other research organisations allowing tissues from each animal to be shared for multiple endpoints. For example, the short term inhalation and oral exposure work will actually be conducted in the Netherlands, and the tissues of the animals divided between institutes in the UK and Sweden. Each institution will measure a different set of parameters. When animal studies are conducted, the numbers used will be kept to a minimum while allowing sufficient data of good quality to be generated to ensure meaningful conclusions can be drawn. Where previous relevant data exists this will be used either as an alternative to conducting animal studies, or to calculate the number of animals needed.
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.	Humans would actually be the best model, and in fact, access to samples from people working in nanomaterial production and handling facilities is being negotiated via a European project, NANoREG. However, this work is limited in scope and so additional models are required. The animal models to be used in this study include rats and mice. The majority of historical data for toxicology and risk assessment studies of nanoparticles, particles and chemicals are generated using these models, allowing

	benchmarking and comparison to a large data set and potentially extrapolation in future. The use of different models would limit such comparisons to be made, slowing down the development of knowledge and increasing the need for further animal studies.

Project 4	**Regulatory Aquatic Ecotoxicology Testing**	
Key Words (max. 5 words)	Aquatic, ecotoxicology, fish, freshwater	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
	X	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)	Substances in the environment, such as pesticides, pharmaceuticals and industrial chemicals, can lead to levels of pollutants in water that can pose a risk to fish, or to humans via the food chain. Most of the work is expected to involve pesticides in the environment. The purpose of the project is to generate data on toxicity of these chemicals to fish. The data generated can be used by the regulatory authorities to assess the environmental risks that are posed by a chemical. In addition, the dietary exposure to humans through eating fish will be measured, which can be used by regulatory authorities to assess and minimise the risk to humans.	
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 project allows an estimate of toxicity of chemicals to fish and to measure uptake of chemicals into fish, to estimate the potential for them to transfer through the food chain. This is used by regulatory authorities to ensure that these chemicals will be within the guidelines for environmental and human safety.	
What species and approximate numbers of animals do you expect to use over what period of time?	Freshwater rainbow trout. Based on recent use, it is expected that up to approximately 800 fish will be used each year.	

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?	Tests that measure the toxicity to fish can produce a range of effects, some of which may be severe. The effects might in include loss of orientation, abnormal swimming, abnormal growth or death. For tests that measure dietary exposure, any adverse effects are expected to be mild, as only very low levels of chemicals are used for this type of test. At the end of testing, animals will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Regulatory requirements for toxicity testing of chemicals require that certain experiments be conducted using specified guidelines and trophic levels. Fish are chosen as model organisms for aquatic environmental risk assessment. It is difficult to replace the use of fish, as no alternatives are currently accepted by regulatory authorities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, the minimum number of animals recommended in the regulatory guidelines will always be used. For toxicity testing, it is often possible to carry out a pilot range-finding study with a reduced number of fish. The results of this can be used to refine the larger test that will be used to generate regulatory data, or to avoid the need to carry out regulatory tests. This use of a pilot study reduces the overall number of fish required for testing. If the data are already available within the EU, the test will not be required. For tests that measure dietary exposure, it is possible to test several chemicals at the same time, with the same group of fish. Where more than one test is being carried out at the same time, where possible they will share one group of control fish (animals that are not exposed to chemical). In some cases, it is possible to use data from non-animal tests (eg algae) to eliminate the need for testing on fish.
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	The fish are observed carefully at regular intervals, with increased frequency during critical phases of the test. In some cases, it is possible to kill fish humanely before the end of a test if they are showing signs of suffering. This can only be carried out if the lab is

measures you will take to minimise welfare costs (harms) to the animals.	certain that the results will still be acceptable, so that there is no risk of a need to repeat the test with more fish. The laboratory continues to discuss this use of 'humane end-points' with its customers. Where fish are used to measure the dietary exposure of humans, the tests are carried out at only one concentration of the chemical, to ensure that as few fish as possible are used.