Home Office

NON-TECHNICAL SUMMARY

Research into the susceptibility of European grayling and pink salmon to infectious haematopoietic necrosis virus

Project duration

3 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

IHNV, Infection, Susceptibility, Listed disease

Animal types	Life stages
European Grayling (Thymallus thymallus)	Juvenile
Pink salmon (Oncorhynchus gorbuscha)	Juvenile
Rainbow Trout (Oncorhynchus mykiss)	Juvenile

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to establish the susceptibility of two fish species to the serious fish disease caused by infectious haematopoietic necrosis virus (IHNV). The pink salmon is a non-native species encroaching on UK waters and the European grayling is a UK native, cohabiting natural and farmed environments with salmonids, so IHNV poses a potential disease threat to both these species and to our wild and farmed salmonid populations.

A retrospective assessment of these aims will be due by 28 May 2028

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

IHNV is a rhabdovirus which can result in high mortality in cultured and wild fish worldwide with high economic losses. The disease is listed by the World Organisation for Animal Health (WOAH) and the UK is currently recognised as disease-free. There is reason to fear that IHNV could still spread to the UK via imports, mechanical transmission or natural migration. Information on IHNV host susceptibility is needed to assess the risks of introduction and transmission between species and will be used to prevent and control future disease incursions.

What outputs do you think you will see at the end of this project?

- New knowledge on the resilience of the European grayling and pink salmon to anaesthetic

- New knowledge on the susceptibility of UK native fish species to IHNV
- This data will inform on policy and risk assessments
- The data will be publicly available through publication in a peer reviewed scientific open access journal/open access report

Who or what will benefit from these outputs, and how?

Pilot studies assessing the welfare and resilience of the European grayling and pink salmon undergoing marking and intraperitoneal (IP) injection under a single period of anaesthesia are intended to minimise harm to the animals under procedure. If a species is suspected to be sensitive to the anaesthetic, by checking a smaller number of fish prior to main study, it ensures the two procedures can be performed sufficiently under one anaesthetic, minimising handling and a second requirement to anaesthetise the fish.

This project benefits fish health management (official control measures) and the aquaculture industry by filling relevant data gaps around susceptibility of fish species to aquatic viruses for prevention and control purposes: 1) to maintain the UK's high aquatic animal health status; 2) to protect aquatic animals from the impacts of diseases; 3) to provide an environment in which the aquaculture sector can operate (by mitigating risks of introducing listed pathogens into the UK/England or their spread); 4) to support UK aquatic biosecurity and to comply with UK and EU legislation on aquatic disease; 5) to support global seafood security. Overall, this benefits the animal populations (susceptible species), people who own or work in the aquaculture sector, recreational anglers and could help to ensure food for a growing world population with an increase in demand for protein.

Lack of data means that significant risks (e.g. risk of disease spread via live fish movements) may not be recognised and international legislation omits species that should be listed as susceptible, allowing trade without conditions on IHNV health status.

The data and publications resulting from this project will be used to inform risk assessments and the competent authority on the associated risks. Positive results would provide evidence to support a case for amendment to UK, EU and the World Organisation of Animal Health lists of susceptible species.

How will you look to maximise the outputs of this work?

Knowledge on the resilience of specific species to anaesthetic will be shared via a peer reviewed open-access scientific journal where relevant to ensure best practice is used when performing procedures under anaesthesia in these species.

Dissemination of new knowledge to the UK governments and the aim to publish results in a peer reviewed open-access scientific journal and report.

Extra tissue or water samples may be frozen or preserved to aid related future research.

Species and numbers of animals expected to be used

- Other fish:
  - European Grayling (Thymallus thymallus): 500
  - Pink salmon (Oncorhynchus gorbuscha): 500
- Rainbow Trout (Oncorhynchus mykiss): 960

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The species chosen are relevant to the infectious disease under investigation. Rainbow trout represent known susceptible species as positive controls. Juvenile fish (<20 g) have been reported to be more susceptible than adults (summarised in the relevant disease chapter published by the World Organisation for Animal Health). At this life stage they are robust to the required handling and will provide enough sample tissues for thorough, diverse analyses of susceptibility.

Typically, what will be done to an animal used in your project?

Susceptibility will be assessed by two routes of infection: (1) Intraperitoneal injection of the virus into the fish and (2) cohabitation of naïve non-injected fish with IHNV injected fish (that have been marked for identification).

Pilot studies:

Under general anaesthesia, the fish will be marked using a coloured implant under the skin for identification and intraperitoneally injected (into the peritoneal cavity) with cell culture medium and returned to an experimental tank. The fish will be observed frequently for up to 30 minutes. After 30 minutes the fish will be humanely killed using a Schedule 1 method.

Main study:

IHNV inoculum will be produced in cell cultures, containing appropriate growth medium, and quantified in cell culture. Under anaesthesia, the fish will be marked using a coloured implant under the skin for identification and intraperitoneally injected with a quantity of IHNV (suspended in cell culture medium) or the same volume of cell culture medium alone. The fish will be returned to their study tanks for recovery. These tanks will either already contain an equal number of naïve cohabitant fish, or an equal number of naïve fish will be added. The fish will be observed for up to 6 weeks. Clinical signs (external appearance and behaviour) will be monitored and defined humane endpoints will be implemented to avoid mortality where possible. At the end of the study the remaining fish will be humanely killed (using a Schedule 1 method or approved non-Schedule 1 method) for postmortem examination and collection of tissue samples for further analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Impacts from injection of the virus and marking are expected to be minor and will be performed under anaesthetic. The fish are expected to fully recover from the procedure within minutes.

Pilot studies with small numbers of animals may be required to ensure levels of anaesthesia are maintained and appropriate for the European grayling and pink salmon if deemed to be sensitive to the anaesthetic. In these cases, animals may be terminated without full recovery.

IHNV is a serious disease of salmonids frequently causing mortality, within approximately 5 days of infection for a virulent isolate. Fish infected with IHNV can exhibit lethargy mixed with bouts of frenzied abnormal activity; reduced feeding; spiral swimming; flashing; faecal trails; darkening of the skin; bulging eyes; a swollen abdomen; and haemorrhaging. On closer examination: fish may have fluid within the abdomen; pale gills, liver, kidney and spleen; tiny pinpoint red marks caused by haemorrhaging; yellow mucus in the intestine; and a lack of food in the stomach. Where fish survive an outbreak, spinal deformities may become evident. Rainbow trout are known to be susceptible so it is likely that they will show one or more of these abnormalities. After first signs of disease, the fish will likely reach a humane endpoint within a day or two. The susceptibility of European grayling and pink salmon is unknown.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

It is expected that: 25% of each species will experience sub-threshold levels of severity (cohabitant negative control fish with no invasive procedure and no adverse effects expected); 25% of each species a mild severity (receiving an invasive procedure (injection) with no adverse effects expected); 40% of each species moderate severity (removed at a defined humane endpoint within 12 hours of previous check); and 10% of each species might reach severe severity.

What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 28 May 2028

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

For a species to be listed as susceptible by the World Organisation of Animal Health, a number of criteria need to be met based on: (1) the route of transmission being consistent with natural pathways for the infection, (2) the pathogenic agent being adequately identified and (3) evidence that presence of the pathogenic agent constitutes an infection. For this we need to use animals. It is important to know if the fish are infected and recover, have the potential to carry the virus without clinical signs, or if the infection could cause mortality.

Viral infection and transmission in an animal is determined by complex interrelated environmental influences, metabolic, anatomical and immunological mechanisms (Figure 1). Non-animal alternatives have not been developed to fully and accurately represent these for different individuals, species, life stages and multiple tissues and cell types. Live animals cannot be adequately replaced with animals at a more immature life stage, species that are less sentient, or animals that have been terminally anaesthetised.

Continual review will ensure replacements are not missed.

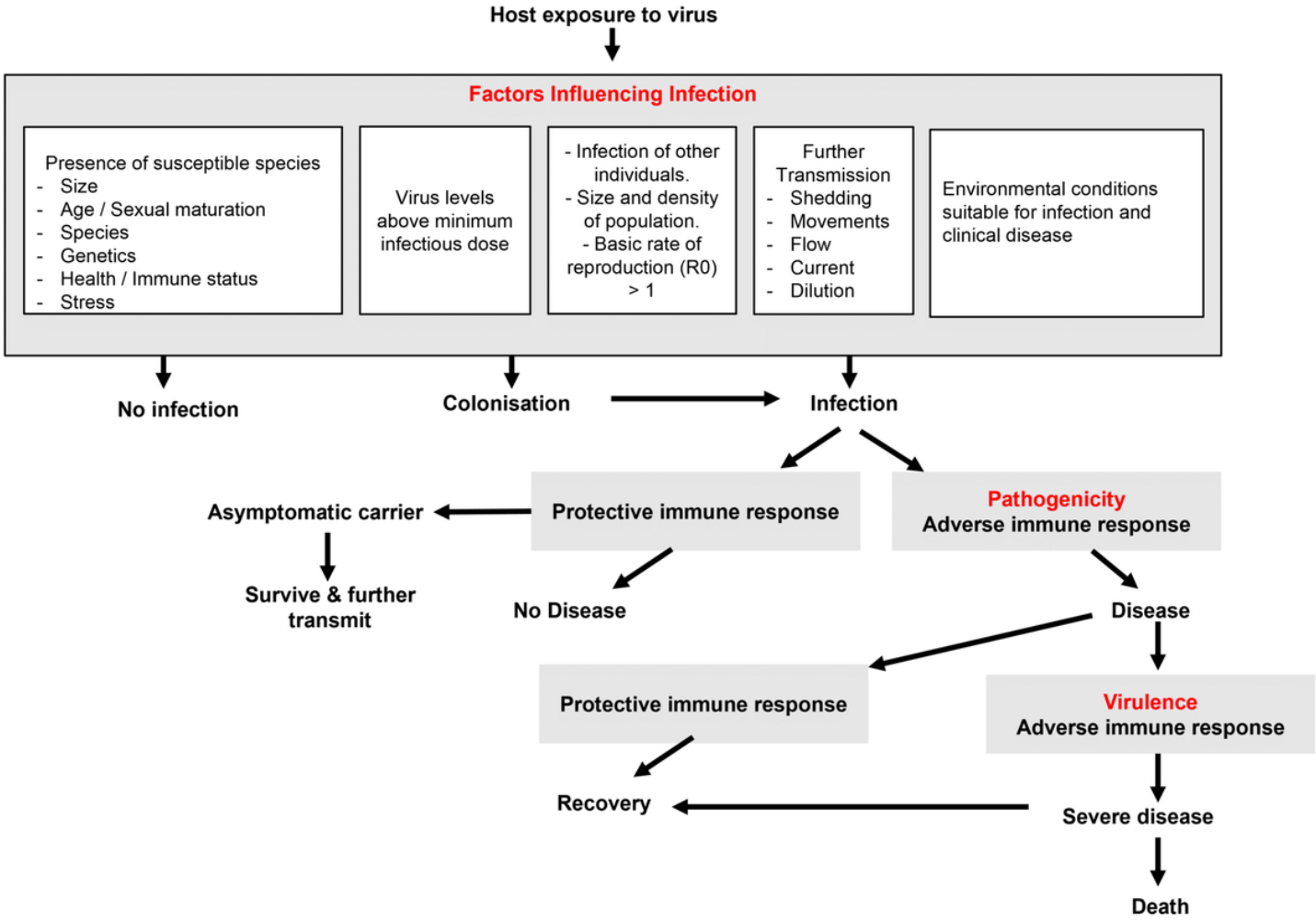

Figure 1. Factors influencing viral infection and transmission in fish

Which non-animal alternatives did you consider for use in this project?

- Non-animal models e.g. *In vitro* cell culture, algae or daphnia.
- Predictive models.
- Non-protected embryo life stages.

Why were they not suitable?

- Cell culture will be used to grow the viral stock and quantify the virus but is not suitable to demonstrate susceptibility of the species. *In vitro* cell culture methods (including 3D cell culture) are not developed enough to represent all species with multiple tissues and cell types and responses. Although useful for studying certain aspects of viral infections, such unicellular systems fall short in creating an understanding of the processes that occur at an integrated tissue level. There are no known non-animal alternatives that can adequately correspond to the complex interrelated metabolic, anatomical and immunological mechanisms during the viral infection and transmission process, (i.e., the route of infection, complex interplay between virus, target cells and immune responses, and the difference in resistance of specific individuals, multiple tissues and cell types, shedding of the virus, and transmission from fish to fish).
- There are no predictive models for unknown susceptibility.
- The infection of embryos has not been sufficiently shown to represent the infection profile of hatched fish of any species. The primary portal of entry of IHNV is considered to be the gills, but tissues of the digestive system may become infected e.g. if fish, particularly juveniles, eat others that have died from the disease. These portals are not present in unhatched eggs or pre-feeding hatched embryos.

A retrospective assessment of replacement will be due by 28 May 2028

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals to be used and minimum number of replicate tanks have been discussed with and approved by our statistician and experienced aquarists. Numbers have been estimated for each of the unknown susceptible species and the number of experimental units, accounting for replication within study, testing one concentration of IHNV. Group sizes are guided by the husbandry requirements of the fish (particularly stocking density) and to provide robust data for statistical analysis.

An example of how our estimates have been calculated is:

Pilot study (optional):

- 10 European grayling and 10 pink salmon VIE marked and IP injected with cell culture media. + repeat (TOTAL = 20 European grayling and 20 pink salmon)

Main study:

- Two tanks of 30 VIE marked and IP injected fish + 30 cohabitant naïve fish for each species. The IP injection will be with IHNV (positive controls in susceptible rainbow trout and unknown susceptibility in European grayling and pink salmon) and IP injection with cell culture medium (negative controls) (Total = 240 of each species per experiment).
- Double the amount of rainbow trout have been estimated (but may not be required) compared to the other species under investigation as two studies may have to be performed independently for European grayling and pink salmon, with controls for each, due to when the fish spawn and are available throughout the year.
- A repeated experiment has been included for each species in case of unforeseen experimental problems, or due to fish health issues unrelated to the study.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Quantification of virus by cell culture will ensure the appropriate amount of virus is sufficient and viable for use in an animal study.

Tools for best practice have been considered such as the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines and fish specific guidelines (NORECOPA) and the Experimental Design Assistant (EDA) for appropriate design of experiments. Animal numbers have been estimated based on the number of experimental units, accounting for replication and using our extensive experience in viral disease models. We plan to use the minimum animal numbers able to provide enough power to detect statistical differences between treatments. Group sizes are also guided by the husbandry requirements of the fish (particularly stocking density). The numbers of animals to be used has been discussed with, and approved by, our statistician and experienced aquarists.

Continual review and improvements will be made where possible to the infrastructure of the facility to enable further reduction of animal numbers with regard to husbandry requirements. An example of a previous improvement at our facility is the design of variable standpipes, which enable stocking density

adjustment by changing the tank volume (i.e. tank volume can be reduced to increase the stocking density most suited for a species rather than using more fish).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

It is unclear how resilient European grayling and pink salmon are to recognised fish anaesthetics. If required, pilot study/studies may be undertaken to refine the procedure by confirming the resilience of the European grayling and pink salmon to undergo visible implant elastomer (VIE) marking and intraperitoneal (IP) injection procedures under a single period of anaesthesia. If the anaesthetic level and duration of anaesthesia was determined to be satisfactory with a single period of anaesthesia, the animals would be marked and injected at the same time. Conversely, if the duration of anaesthesia required to undertake both VIE and IP injection under a single period of anaesthesia was disproportionately harmful, then marking and IP injection would occur separately under sequential periods of anaesthesia with an appropriate recover period between events. The advice and guidance of the Named Veterinary Surgeon and Named Animal Care and Welfare Officer will be sought and applied during any pilot study determining appropriate levels of anaesthesia.

To reduce variation, animals within a study will be ordered in batches from the same supplier and be of similar age / size. Previous mortality data from IHNV experiments will be considered to ensure a successful challenge. Samples will be taken for multiple analysis routes. All fish will either be frozen or preserved if they are not analysed immediately, allowing for later analysis or made available to other researchers for associated research. For example, these samples may be used on future research (funding pending) to study host response through RNA-Seq analysis to get a better understanding on the molecular mechanisms of infection among susceptible and non-susceptible species. This knowledge will aid on designing control measures such as resistant breeding selection and vaccine development. Water samples may also be used for studies researching environmental DNA (eDNA) detection.

A retrospective assessment of reduction will be due by 28 May 2028

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The fish may be produced in our own breeding establishment (from eggs brought in) to ensure disease-free status and high-quality animals. In cases where we need to purchase juveniles from fish farms, we typically select sites with an established health history and high standards of biosecurity and animal welfare (including during transportation). Externally sourced juveniles will be held in quarantine while a health screen takes place to ensure a good health status, and acclimation to our tank conditions, before use in studies.

We will abide by published guidelines and ensure best working practice. Husbandry information (including environmental enrichment, e.g., artificial weed or hides, social, spatial and visual enrichment) is obtained for all species to inform the species-specific needs and ensure they are met. Advice is sought from published literature, experienced Fish Health Inspectors, experienced aquatic facilities staff, the Named Veterinary Surgeon and the Named Animal Care and Welfare Officer.

The two novel species have been chosen due to their current unknown susceptibility and perceived potential risk of introducing the virus to susceptible species native to the UK. Rainbow trout are susceptible species so having these as a positive control for infection is vital to interpretation of results. Juveniles will be used as the most susceptible life stage.

To find out if the species are susceptible, we need to observe their appearance and behaviour post challenge and observe for any infection with recovery. Holding up to 6 weeks after challenge will allow for observation and sampling throughout. Humane non-Schedule 1 killing will allow samples to be taken of better quality for analysis.

Fish under procedure will be monitored via direct visual checks using standardised in-house record sheets for abnormalities. It is not possible to maintain large numbers of fish under anaesthesia for the duration of the study which would also interfere with natural transmission. Additional pilot studies may be undertaken to ensure appropriate levels of anaesthesia and duration of anaesthesia are applied to minimise handling and stress.

Early and humane endpoints will be used, when possible, to minimise suffering while still providing valid results. The frequency of direct visual checks will increase when adverse effects are expected or present, with frequency related to the severity and speed of progression of clinical signs. Direct visual checks will be supplemented by video observations using underwater cameras mounted within tanks, when possible, to observe behavioural and morphological changes not easily detectable during a normal direct check. The facility has over 30 cameras used routinely in disease challenge work. As an aid to implementing humane endpoints the cameras have been invaluable in our ability to refine severe Protocols.

Why can’t you use animals that are less sentient?

More immature life forms/less sentient species, for example algae, daphnia, fish embryos or invertebrates, are not a suitable replacement due to the great differences between virus-host interactions, species susceptibility and sensitivity at different life stages.

Some fish species reach first feeding very soon after hatching, for example grayling first feed 4-5 days after hatch, so the experimental period would extend beyond the protected stage. It is important to know if the fish are infected and recover, have the potential to carry the virus without clinical signs, or if

the infection could cause mortality. First signs of disease are expected after approximately 5 days and progression of the disease, shedding of the virus, further transmission to naive cohabitant fish, or recovery would not be possible in immature stages before first feeding.

Juvenile fish are reported to be the most sensitive stage to indicate susceptibility and will be used to provide clear results. The infection of embryos has not been sufficiently shown to represent the infection profile of hatched fish for any species. The primary portal of entry of IHNV is the gills, but tissues of the digestive system may become infected e.g. if fish that have died from the disease are eaten by others - this is not possible in unhatched eggs.

Terminally anaesthetised animals would not have the same behaviour, metabolic mechanisms or responses to an infection.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Rainbow trout are known to be susceptible to IHNV and will act as a positive control to support the interpretation of results and confirm the virus as infectious. As susceptibility is unknown for grayling and pink salmon, expected adverse effects may be severe. Early and humane endpoints will be used, when possible, to remove fish before this severity is reached. Standardised in-house record sheets will be used to record abnormalities in behaviour or appearance over time (where one observation provides a snapshot, while the development and duration of clinical signs are important additional considerations). If required, small (pre)studies will be undertaken to minimise handling and determine optimal anaesthetic dose during marking and IP injection to minimise cumulative harm.

Frequent monitoring will be undertaken to ensure humane endpoints to avoid mortalities - at least twice a day, increasing in the period during and directly after treatment and if clinical signs of disease are observed or expected. The frequency of direct visual checks will be related to the severity and speed of progression of clinical signs. The frequency will be at an appropriate level that avoids or minimises harm between checks, so that no animal will be subjected to prolonged suffering.

Where possible remote monitoring via a camera will be used to monitor the fish. Video surveillance will be dependent on the number of available cameras and tank design, but where possible will aid in observation of behavioural and morphological changes not easily detectable during a normal direct check.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Tools for best practice for design of experiments will be referred to, e.g.: the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines (http://journals.sagepub.com/doi/full/10.1177/0023677217724823); fish specific guidelines (NORECOPA) (https://norecopa.no/prepare); and the Experimental Design Assistant (EDA) (https://www.nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda). Other resources include guidance and publications from the NC3Rs (https://nc3rs.org.uk/3rs-resources) and the CCAC (https://ccac.ca/en/guidelines-and-policies/the-guidelines/).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep up to date about advances in the 3Rs by regularly checking information on websites (e.g. NORECOPA, NC3Rs), attending scientific meetings, RSPCA meetings/publications, peer reviewed scientific publications, communication with other scientists and obtaining advice from the Named Persons.

By regularly checking the internet we will keep up to date with related publications and updates to the lists of susceptible species.

We are continually refining systems further updating the infrastructure needed for procedures involving protected animals.

A retrospective assessment of refinement will be due by 28 May 2028

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?