PLEASE CIRCULATE TO ALL LICENCE HOLDERS AND OTHER ROLE HOLDERS IN YOUR ESTABLISHMENT

Retrospective Assessment

Following the implementation of the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012, the Secretary of State is required to undertake the Retrospective Assessment (RA) of certain projects granted since 1st January 2013. Projects entailing the use of non-human primates, dogs, cats, equidae and endangered animals, procedures on any species that have been classified as severe, and projects for the purpose of education and training will be required to undergo RA. ASRU has now formalised the process to achieve this.

In order that the RAs of licences are carried out in a consistent manner, and for everyone involved to have a shared understanding of the process, please find attached preliminary instructions for establishments which sets out a list of key points, frequently asked questions and an example completed template.

We trust this will be helpful but would welcome any feedback, including further questions or points for clarification. Please email these to the ASRU Business Support Team (ASRUBusiness@homeoffice.gsi.gov.uk) and write Retrospective Assessment in the subject line.
Instructions for establishments: Retrospective Assessment

Background

Retrospective Assessment (RA) is a legal requirement that needs to be undertaken on specific types of project licences that have been granted under the Animals (Scientific Procedures) Act (ASPA). This requirement is applied to certain project licences granted since 1 January 2013. The aim of the RA is to determine: whether the objectives have been achieved; the harms caused to animals; the number and species used; the severity of the procedures; and, whether lessons can be learnt to further the implementation of the 3Rs.

Project licences entailing the use of non-human primates, dogs, cats and equidae, the use of procedures classified as severe, endangered animals or for the purpose of education and training will have RAs applied to them.

The Home Office will publish the RA reports alongside the non-technical summary (NTS) that is already published.

This communication outlines the process and some key points to help establishments undertake their part in gathering the information required for the completion of the RA.

The Retrospective Assessment Process

A flow diagram of the RA process is attached at Annex A. Key parts for establishments are in yellow.

The RA template with advice to project licence holders for completion is provided at Annex B (a). An example of a completed RA template is at Annex B (b).

The following process will be used for RAs:

1. When the licence is granted by the Home Office, or following an amendment that requires a RA, a statement shall be applied to the project licence explaining that a RA is required, (on the ‘red seal’ page if you have a paper licence).
2. The default position is for all RAs to be due at the expiry of the licence in question.
3. The project licence holder will be reminded of the need to undertake an RA in the 12, 6 and 3 month expiry reminder letters issued by ASRU. This letter will contain a template for completing the summary information.
4. The licence holder will need to gather the necessary information required for their local AWERB to undertake a review of the project paying particular attention to the progress made to meet the objectives of the project, the harms experienced by the animals and benefits accrued, and any 3Rs lessons learned.

5. The licence holder must then complete the template by summarising this information and submit the completed template to ASRU (currently via the establishment’s Single Point of Contact (SPoC)).

6. The assigned inspector will assess the information submitted and complete their report on the RA. The report will confirm whether the information submitted is a reasonable reflection of the progress made in the project, determine whether the harms and benefits reasonably reflect those stated in the original application (including any unexpected harms or benefits) and highlight any 3Rs lessons learned during the project.

7. The inspector may need more information from the licence holder and will contact them directly if this is the case.

8. ASRU will then publish the summary information in the completed template together with the report from the assigned inspector, alongside the original NTS to form the updated NTS. The RA will be published, normally within three months of the expiry of the project.

9. Confidential information, including personal information, commercial or intellectual property must not be included in the template since the RA will be published.

ASRU will review the operation of this process after 12 months. To contribute to the review, ASRU would value your feedback. Please email your feedback to the ASRU Business Support team at ASRUBusiness@homeoffice.gsi.gov.uk and put Retrospective Assessment in the subject line.

Points to note

- If a new project, or an existing project submitted for amendment, requires RA, ASRU will identify this during the assessment process. If an RA is required, the resulting licence will clearly indicate whether, and at what time, it is required to be carried out.
- ASRU will set the date to undertake the RA at the expiry of the licence. This is so the majority of the work will have been undertaken by the time the RA takes place and a full picture of the objectives met and benefits accrued, together with any 3Rs advances made, can be obtained. In practice, the licence holder will gather the information required for consideration by the AWERB several months prior to the expiry of the licence in order that there will be sufficient time for the AWERB to conduct its review and for its summary to be submitted to ASRU in good time for the report of the RA to be written and published.
- ASRU will send a reminder letter to the licence holder 12, 6 and 3 months before the RA is due to be undertaken.
- The reminder letter will contain the template for the licence holder to submit the required information to ASRU.
Your assigned inspector will consider the information you have submitted and in doing so confirm whether the information is a fair reflection of the programme of work undertaken under the authority of your project licence, comments on the actual harms and benefits accrued so far and any lessons learned regarding the 3Rs (3Rs opportunities identified and implemented). The RA will then be published alongside the licence’s NTS, to form the updated NTS.

ASRU will additionally check the information you supply in a similar way to the NTS i.e. remove personal information that may identify the licence holder or the establishment.

Frequently Asked Questions

1. Q Is Retrospective Assessment the same as Retrospective or Interim Review?

   A No. Retrospective or Interim review of project licences is a task undertaken by AWERBs on all projects being undertaken at the licensed establishment (see section 10.4(d) of the Guidance to the Act). Retrospective Assessments are undertaken by the Secretary of State, with the assistance of the licence holder, the establishment’s AWERB and the assigned inspector.

2. Q The severe protocol has not been used – do we still have to carry out a retrospective assessment?

   A Yes. This is because the RA is carried out on the programme of work in its entirety not just the protocol with the severe severity category. It is possible that the licence holder has been able to complete the programme of work without the use of the severe protocol and this might represent a 3Rs advancement.

3. Q The information provided by the licence holder refers only to the severe protocol – is this acceptable?

   A No. This is because the RA is on the entire programme of work and the severe severity protocol will be an integral part of that programme of work.

4. Q The licence holder has returned all the animals on the severe protocol as moderate – do they still have to provide the information for the RA?

   A Yes. This is because experience and perhaps careful preparation, advice taken from named persons or use of an alternative method has resulted in a lower level of actual severity than the prospective severity. This may be an important 3Rs advancement that can be captured and disseminated.

5. Q I thought the NTS had to be updated to incorporate all the information to be supplied by me to complete the RA – is this right?

   A No. The current version of the NTS should not be revised. The assigned inspector will create a report based on information requested. The information we require will be asked for in the form sent by the Licensing Team. It is this
information that will be reviewed by the AWERB and submitted to ASRU for consideration by your assigned inspector. This ASRU report will be published alongside the NTS. The RA report plus the NTS becomes the ‘updated NTS’.

6. **Q** My project licence requires more than one RA at various times but the default position is for one RA at the expiry of the licence. What should I do?

   **A** If more than one RA is required for your project you need only submit one RA at the expiry of your licence. If you have submitted information already, you may use this to help construct the RA at the end of the project. Please contact the Head of Licensing if you have any queries regarding this.
Appendix 1 Process Map for Retrospective Assessment

**Application for a Project Licence**

- **Project Assessment**
  Inspector determines if criteria met for RA
  
  Inspector recommends RA required

- **Project Licence Granted**
  ASRU reminder Letter sent to PPL Holder 12 months prior to expiry. (Additional reminders sent at 6 and 3 months)

- **PPL Holder supplies the necessary information to the establishment’s AWERB for review**

- **Assigned inspector reviews information, requests further information as required and completes report on the RA**

- **RA report passed to ASRU for publishing**

- **NTS is updated by adding the RA and report (3 months following expiry)**

- **PPL Holder submits the necessary information to ASRU Licensing Team (SPoC) at the expiry date**
## Appendix 2 Template for Retrospective Assessment

### A. Template with advice for completion

<table>
<thead>
<tr>
<th>PART A: FOR OFFICE USE ONLY – NOT FOR PUBLICATION</th>
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<tbody>
<tr>
<td><strong>Project Licence Details</strong></td>
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<tr>
<td>PPL Number</td>
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<tr>
<td>Title</td>
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<tr>
<td>Holder</td>
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<tr>
<td>Date of PPL expiry</td>
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<tr>
<td>Date information submitted by PPLH</td>
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<tr>
<td>Date RA completed by Inspector</td>
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### PART B: Annex to the Non-Technical Summary – TO BE PUBLISHED

<table>
<thead>
<tr>
<th>Information required for Retrospective Assessment</th>
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<tbody>
<tr>
<td>Describe to what extent the programme of work has been carried out</td>
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<tr>
<td>Please state whether the programme of work has been completed or if it is to be continued under the authority of a further project licence if necessary</td>
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<tr>
<th>Describe if, and to what extent, the objectives of the work have been achieved?</th>
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<tr>
<td>Please relate this section to the stated objectives in the original application (including any amended or additional objectives as necessary)</td>
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<tr>
<th>Describe the actual harms that have been caused to the animals (number, species, severity)</th>
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<tbody>
<tr>
<td>Please describe the harms in terms of your reflective assessment of the pain, distress, suffering, or lasting harm you consider the animals have experienced. Do not simply list the procedures applied but describe the harms in terms of animal based outcomes e.g. animals experienced mild discomfort as subcutaneous tumours were allowed to grow to around 10mm in diameter before they were humanely killed.</td>
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<thead>
<tr>
<th>Describe what lessons, if any, have been learned that contribute to the 3Rs.</th>
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<td>Please describe any 3Rs opportunities identified and implemented.</td>
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<table>
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<tr>
<th>Replacement</th>
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<td>Reduction</td>
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<td>Refinement</td>
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### B. Template with example text

<table>
<thead>
<tr>
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<td>v1 April 2018</td>
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### PART B: Annex to the Non-Technical Summary – TO BE PUBLISHED

**Information required for Retrospective Assessment**

**Describe to what extent the programme of work has been carried out**

Studies of putative adult cardiac stem cells in mouse myocardium has successfully identified cells with robust clone-forming ability and multi-lineage potential that are able to convert into cardiac muscle, vascular smooth muscle, or endothelium after grafting to the heart, and which improve cardiac pump function.

**Describe if, and to what extent, the objectives of the work have been achieved?**

Major achievements relating to this BHF- and MRC-funded work include: Pinpointing which among the many classes of cardiac stromal cell has reproducible stem cell properties; defining the cells' molecular signature by single-cell qPCR and their embryological origin by Cre/lox fate-mapping; systematically investigating the properties of cloned adult cardiac stem cells (single-cell derivatives) and proving their fidelity to freshly isolated cells; showing that stem cell grafting helps prevent heart failure after experimental myocardial infarction, even though just 10-20 cells engraft durably; demonstrating that signals secreted by the adult cardiac stem cells can reduce human cardiac muscle cell death, using simulated myocardial infarction in tissue culture.

In addition, our Wellcome Trust-funded drug discovery programme to create small-molecule inhibitors of enzyme of interest has achieved: determining the compounds' pharmacokinetics and establishing conditions suitable for proof-of-concept testing in vivo; proving that this enzyme inhibition reduces infarct size by roughly 70%, if given prophylactically before ischemia-reperfusion; proving, further, that enzyme inhibition reduces infarct size by roughly 70%, even if given two hours after injury, reducing muscle cell death from both apoptosis and necrosis.

**Describe the actual harms that have been caused to the animals (number, species, severity)**

The principal harms arose due to surgery to induce a heart attack. This was performed under general anaesthesia with post-operative analgesia to minimise the pain. However, animals will have suffered some distress due to the anaesthetic and some pain, despite pain killers being given, in the days following surgery. Mild and transient pain was caused by taking blood samples or injecting a medicines to help improve the function of the heart. All animals were humanely killed at the end of the experiment under deep anaesthesia following removal of the heart for further examination in the laboratory. A smaller proportion of animals underwent an aortic banding procedure to induce a different kind of heart failure. The surgery was performed under anaesthesia with post-operative pain relief. The animals will have experience some pain and discomfort in the days following surgery. Again mild and transient pain will have been experienced when blood samples were taken or medicines administered. One animal suffered abnormal breathing for about 14 hours. Other animals...
did not appear to have breathing difficulties as a consequence of the heart failure. In both protocols 10% of animals showed signs of prolonged sedentary behaviour and ruffled fur in excess of that expected within the 24 hours and so were humanely killed. One animal was found dead due to cardiac rupture. When cardiac rupture occurs, we believe that unconsciousness occurs very quickly due to sudden loss of blood pressure and so harms are thought to be of very limited duration

Describe what lessons, if any, have been learned that contribute to the 3Rs.

Replacement

We are continuously doing some literature and web searches (Google, pubmed.com, nc3rs.org.uk) in an effort to find alternatives to any procedure that causes more than momentary pain or distress

Most work (e.g. myocardial infarction, stem cells injections, echocardiography, invasive hemodynamic measurements) requires an intact functional heart. However replacement has been possible in part of the work (e.g. cardiac cells protection/regeneration) with the cell culture approach.

Tissues/organs are frequently collected from animals killed by schedule 1 methods from most of our mouse lines. Samples of whole heart, lung, bone marrow, and occasionally liver, blood and skeletal muscle are used to maximise the information we can obtain from each mouse, particularly when it does not involve additional welfare cost to the animal. The tissues obtained are used in a broad range of downstream investigations, e.g. protein analysis, infarcts staining and histology, gene expression to aid our understanding of disease mechanisms taking place in our mice models of cardiac disease.

In cell graft experiments, only grafting to the intact heart itself is an adequate model to facilitate potential human trials of cell therapy for cardiac repair, and only grafting to the injured heart is an adequate model to ascertain the cells potential differentiation and functional impact in a clinically relevant scenario.

Reduction

Using the latest imaging technologies, e.g. cine-magnetic resonance (MRI) – including late gadolinium enhancement (LGE) imaging- we strive to maximize the information obtained from each animal during the same imaging study. Detection of subtle cardiac changes in mice treated with new compounds which would potentially be missed by conventional imaging and by accurately detecting changes in infarct size and cardiac haemodynamics and reducing the ‘noise’ in the dataset, this allows us to use fewer animals than before to identify differences between the treated group and the control group.

Refined use of primary cell cultures, the number of mice being required to be humanely killed to generate clones has been reduced from 30 to 10 by incubating cells in ‘hypoxic conditions’ to improve cloning efficiency. The number of cells needed for our analytical procedure has been reduced by the establishment of robust methods for single-cell measurements of gene expression.

Refinement

Myocardial infarction

Surgery is conducted using aseptic technique and physiological support such as supplementary heat and fluids. Analgesia is provided routinely before and after surgery.

The majority of deaths associated with the surgical induction of myocardial infarction occur in the intra-operative period whilst the animal is still under general anaesthesia. Careful monitoring is applied post-operatively to identify key clinical signs and minimise suffering associated with adverse effects. Where these occurred, score sheets identified the extent and timing so that early endpoints could be applied and thereby avoid animals being found dead.
Post-operative mortality is principally due to cardiac rupture. Since this occurs more in male mice, the risk is partly mitigated by use of female mice for this model.

Gene induction

Pilot studies have been undertaken in small numbers of animals to determine the lowest effective dose of tamoxifen to induce genetic alteration in a conditional knock-out affecting cardiac cells. Information from this study in conjunction with imaging studies, should enable safer and effective gene ‘knock out’.

Inspector Report (on behalf of the Secretary of State)

This is my report of the Retrospective Assessment for this licence.

- I am content that the information above accurately reflects the progress made in this licensed programme of work.
- The actual harms caused to the animals have been broadly in line with those predicted at the time of assessment. However, it is noted that some of the severe protocols have not been used due to the work being focussed on one particular disease model thus avoiding a number of animals from suffering severe harms.
- The benefits accrued so far centre on important information on a potential new treatment based on stem cells for heart attack. New knowledge has been acquired and disseminated through publication of experimental results in peer reviewed scientific journals. It is likely that further similar publications will follow in the next year or so from this programme of work.
- A key refinement has been the improved anaesthetic and analgesic regimens for the surgical induction of heart attack. Together with improvements in peri-operative care, the numbers of animals requiring to be humanely killed or being found dead following surgery has been reduced.
- A key replacement alternative has been the use of isolated hearts in vitro to obtain preliminary data on the potential therapeutic use of different cell matrices to enhance the treatment of heart attacks.
- The refinement and replacement alternatives above, together with a focus on a particular model over another has reduced the overall numbers on animals required to meet the scientific objectives in this programme of work by approximately 20%.

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