Retrospective Review of Project Licences

Project Licence Retrospective Review

"The overall purpose of retrospective review in the general sense of '*following the development and outcome of projects'* is, wherever possible, to reduce the harms and increase the benefits of every project at an establishment. The aim is to improve both animal welfare and the quality of science and to help inform future debate on these issues." [RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)]

At the University of Cambridge one (or possibly more) of the following will determine when your licence will be requested for review:

a. 12 to 6 months before your existing licence is due to expire if you have no plans to write a new licence;
b. 15 to 12 months before your existing licence is due to expire if you plan to write a new project licence;
c. 3 months before you terminate your licence if you intend to revoke your licence before its due expiry date;
d. When there is a change of project licence holder (in this case the person currently holding the licence should complete the retrospective review before the amendment is made to change the project licence holder);
e. As determined by the AWERB Standing Committee. The AWERB Standing Committee may decide a licence should be reviewed at a specified time or times based for example on the severity of the work, novelty of the models etc..

The information you provide in or attached to this document must reflect data generated only from work carried out under the licence under review. Please do NOT copy and paste text that appears in your current licence unless requested to do so.

You are not restricted by the size of the text boxes on this form; they are designed to expand to accommodate your text. However please remember that AWERB Standing Committee members, which will include your colleagues, have to review the information you provide so please be informative and concise and do not include information that is not requested. Please remember to use Plain English, where possible.

Please be aware that Retrospective Reviews, as with your current and previous project licences, can be requested under the Freedom of Information Act 2000. It is therefore wise to take care when completing this document and ensure that it does not contain typographical errors, personal details or any sensitive information pertaining to Intellectual Property.

A copy of the final agreed version of this document and any advice provided by the University AWERB in relation to this document will be forwarded to your local Home Office Inspector.

If you use abbreviations could you please ensure the first time the abbreviation is used that it is spelt out fully with the abbreviation in brackets.

Finally, if Home Office has identified your licence as one that required a Retrospective Assessment please still complete this template. The information gathered will be used by AWERB to undertake their required task to review your work and can be used to complete the Home Office Retrospective Assessment template. The latter can be achieved by copy and paste will a further update just before submission to include work you have complete between when you fill in this template and the expiry of your licence, which is when your Retrospective Assessment will need to be sent to the Home Office.

Thank you.

1. General Information:

Project licence holder to complete if information provided is incorrect

Name:	Prof Stefan Marciniak	
Contact details: Address & tel.	Cambridge Institute for Medical Research Keith Peters Building, Biomedical Campus, Hills Rd, Cambridge CB2 0XY	**Email:** sjm20@cam.ac.uk **PA:** Anne Francis af640@medschl.cam.ac.uk
Project No.	PP5789170	
Project title	The integrated stress response in respiratory disease	
Project purpose	We aim to identify components of this "integrated stress response" that when targeted with drugs can treat lung diseases	

Date licence was granted	9th September 2021
Date of retrospective review is sent to the PPLh	10th June 2025

The following are the triggers for licence Retrospective Review:
If you are completing this form because you have been asked to complete a Retrospective Review and our UBS staff have completed this box for you. Please check that you agree with the selection made and correct if you believe the information is incorrect.

A		My licence is due to expire in 6 to 12 months time and I have no plans to write a new licence
B	x	My licence is due to expire in 12 to 15 months time and I plan to write a new project licence to continue my research
C		I plan to request the revocation of my licence before its natural expiry date
D		I will no longer be the project licence holder but another person will continue to hold the licence. **The name of the new licence holder will be**:
E		I took over this licence from (insert name) in (insert date)
F		The AWERB Standing Committee has asked for this Retrospective Review.

Note to licensees:
In the case of **option D** if the new project licence holder's name has not been filled in please add this information to this box.
In the case of **option E** please insert the name of the person from whom you took over this licence and the date when you became licence holder.

2. Animal Welfare/Recorded Harms:

2.1 Using the table below please provide the information requested.
How to complete this table: Return of procedures count animal numbers from 1st January to 31st December. In the table below if your licence was granted part way through the year count year one as the time from when the licence was granted to the 31st December of that year. Year two will be from January to December etc. until the final year for which the report is requested and count from 1st January to the requested report date. You can insert rows as necessary. For any animals that experienced a higher level of severity than that of the protocol please highlight these figures in **bold.** The figures provided can be obtained from your Home Office returns.

Protocol No.	Species	Protocol severity category	Total est. number	Actual number used	Actual severity recorded					
					Non-recovery	Sub Threshold	Mild	Moderate	Severe	Totals
1	Mice	Mild	100	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
2	Mice	Moderate	100	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
3	Mice	Moderate	20	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
4	Mice	Mild	2000	1st year			1			1
				2nd year		134				134
				3rd year		65	96		**1**	162
				4th year		42				42
				Total		241	97	0	1	340
5	Mice	Moderate	1000	1st year				29		29
				2nd year				52		52
				3rd year						
				4th year			168			168
				Total		0	168	81	0	249
6	Mice	Moderate	500	1st year						
				2nd year						
				3rd year						
				4th year						
				Total						
7	Mice	Moderate	1000	1st year						
				2nd year			34			34
				3rd year			29			29
				4th year						
				Total		0	63	0	0	63
8	Mice	Moderate	500	1st year						

				2nd year				31		31
				3rd year				99	**1**	100
				4th year				119		119
				Total		0	0	249	1	250
									Whole Total	902

*If actual severity data has not been formally assigned at the point the Retrospective Review is undertaken, the columns can be used to indicate the estimated severity for each of the categories.

2.2 Does the information provided in the table reflect the projected incidence of harm provided in the adverse effects for each protocol? If not please indicate where this was not the case and whether the projected level of suffering was more or less than you anticipated when you first wrote the licence. If the severity category for a protocol was amended during the tenure of the licence please include this information in the reasons box.

Protocol number	Severity higher/ same/ lower than anticipated	Reason
N/A	N/A	In general, the projected incidence of harm was within the anticipated limits when the licence was first written (serious adverse harms of 0.22% over 5 years).

2.3 Describe any unexpected adverse effects and include the percentage and the number of animals affected. How were these episodes managed and recorded? If any of these resulted in a Project Licence Standard Condition 18 report please indicate which were reported under Condition 18 and the date (also see Section 5.2).

Two unexpected deaths occurred.

The first was a breeding female (MKAC16.4i) which was found dead (18 weeks) with no apparent cause found on necropsy. This was reported under a Standard Condition 18 Form on the 11th April 2023 (attached).

The second was a mouse which was found moribund a few minutes after an LPS injection was given as part of a study plan under experimental protocol 8 ('phenotyping and pressure monitoring in induced models of pulmonary hypertension') and was culled using Schedule 1 methods immediately. On necropsy an occult kidney infection was found and it was felt that this was the cause of sudden deterioration. This was reported under a Standard Condition 18 Form (CASE ID 232952) on the 26th Jan 2024 (attached).

2.4 What percentage and number of animals had to be killed or died unexpectedly before the end of the experiment? Please provide a brief summary of each incident by protocol(s) where this occurred.

Protocol No.	% and number of animals killed or unexpectedly found dead
4	1 animal unexpectedly found dead (0.29%)
8	1 animal culled before the end of the experiment (0.4%)

2.5 Were your estimated animal numbers accurately reflected in your actual animal usage as provided in Table 2.1 above? **NO**

If no, please explain the why there was a difference. Please do this on a protocol by protocol basis.

Protocol No.	If your numbers were less that estimated please explain why.
	Our animal usage was significantly less than the estimated animal usage in all protocols. The reasons for this were (i) experimentation was significantly curtailed by the SARS-CoV-2 pandemic; and (ii) the main experimentalist had a family member with a terminal illness during this period and so most of the work planned was carried out by a collaborator instead.

2.6 Have you re-used animals? **NO**

If so, why have you re-used animals and what has been the impact of re-use on the welfare of your animals? Please also provide the species and number of animals re-used by protocol where re-use occurred.

Species	Protocol No. on which animals where/will be returned as re-used, why and what impact, if any, this re-use had on the welfare of your animals

2.7 Has there been any animal wastage? **YES**

For example, this may occur when breeding and maintaining genetically altered animals or when animals have been ordered and cannot be used for some technical reason.

Where wastage has occurred, please explain the circumstances and nature of the wastage plus an indication of the numbers of animals. Please also provide either the number or percentage of animals that were wasted.

Circumstances and nature: A 'double knock-out' line was created, which necessarily meant that larger numbers of animals with undesired genotypes were created since the breeding of 2 double heterozygotes necessarily creates the double knock-out genotype in only 1 of 16 animals.
Estimated number or percentage of animals wasted and if appropriate provide this information by protocol: Protocol 4: 186 mice, 35%

2.8 For licences that authorise the breeding and maintenance of genetically altered animals:

a) explain how you optimise breeding efficiency, and
b) explain what use is made of any excess stock animals, and
c) explain if, and how, you have used any transgene repository – national, international, or internal.

a) Breeding efficiency is optimised by choosing optimum breeding strategies. If the line is not anticipated to be used imminently, we will breed as pure KO colonies (if this has been shown not to be harmful) to minimise wastage.
b) Excess stock animals are offered and given away to fellow researchers in the university if possible. There is an internal e-mail list in which researchers may request access to a particular strain.

2.9 If you breed genetically altered animals are your strains recorded on a database (e.g., MCMS) that can be

a) accessed by other researchers at the University? **YES**
b) accessed by researchers outside the University? **NO**
c) would you allow other researchers at the University to use your strains? **YES**
d) would you allow external researchers to use your strains with an MTA applied? **YES**

If you have answered YES to any of these questions please elaborate below:

a) Colony management and strains are routinely recorded on MCMS.
b) N/A
c) Excess genetically modified animals are routinely offered to/shared with collaborators and other researchers within the university.
d) We have previously shared animals with a collaborator from the University of Sheffield under a MTA.

If you have answered NO to any of these questions please explain why.

b) For reasons of security researchers outside the university are not allowed free access to MCMS.

2.10 Have you been able to refine and/or reduce the impact of contingent harms arising from your use of animals (contingent harms are harms for example that arise from the transportation of animals, single housing animals or housing animals in non-Code of Practice accommodation, etc.). **NO**

Please explain/provide examples.

2.11 By species and percentage please indicate the source of your animals.

Species	Source	Approx. percentage
Mouse	Bred in-house	100%

2.12 During the tenure of the licence have you requested an amendment to increase/decrease animal numbers? **NO**

If so which protocols were amended and were numbers increased or decreased, and by how much?

Protocol No.	Original estimated Number	Amended estimated number

If you have increased or decreased animal numbers please explain why this was necessary?

Not applicable

2.13 During the tenure of this licence have you requested an amendment to increase/decrease the severity of any protocols? **NO**

If so which protocols were amended and were the severity categories increased or decreased?

Protocol No.	Original severity category	Amended severity category

If you have increased or decreased the severity category of any of your protocols why was this necessary?

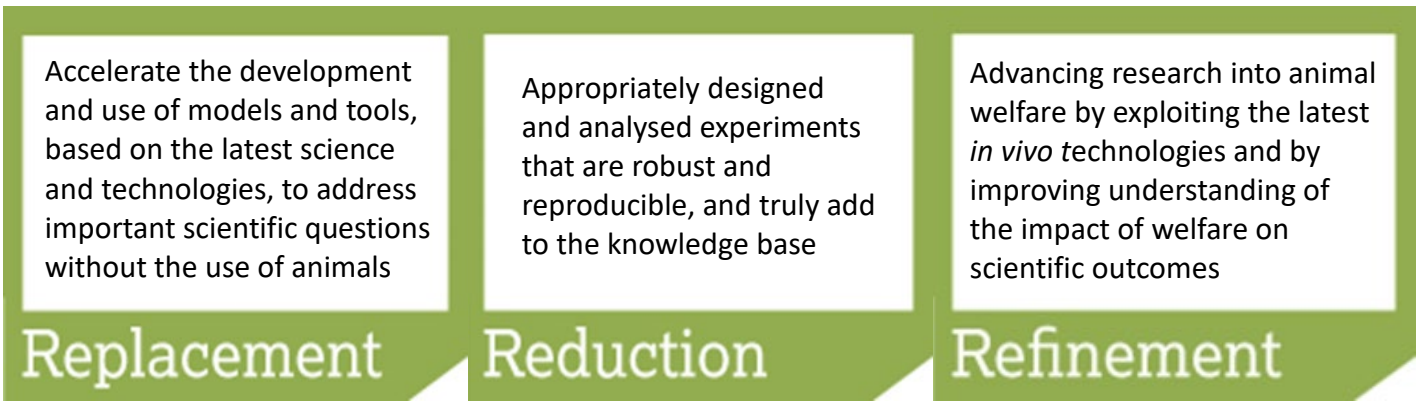
Not applicable

3. **3Rs:**

In this section, please explain what steps you have taken during the tenure of your licence that resulted in 3Rs gains. This might include working with collaborators to refine your model, changing the anaesthetic regime or equipment, etc. Consider every change you have made and its impact on the 3Rs – even small changes and initiatives can result in 3Rs gains.

The following definitions come from the NC3Rs website and are included to help you provide information under the most appropriate R below:

Definitions



3.1 Have you attempted or been able to replace any of your techniques or models with non-animal alternatives? **NO**

Please explain.

Mice are necessary as a major part of our mission is to modulate/improve cardiopulmonary phenotypes which are lacking in fish or *Drosophila* models.

3.2 Have you been able to reduce your animal usage? **YES**

If so, how have you achieved this (e.g., through experimental design, changes to the regulated procedures used)? Please give an indication of the actual numbers reduced.

We have used a new breeding protocol to reduce wastage while generating our double-knockout mice; and have proceeded from getting 2 double-knockouts per breeding pair per quarter-year to 6 or 7 per breeding pair per quarter-year.

3.3 Are you involved with any tissue sharing either within the University or more widely? **YES**

Please explain:

We have both received and donated mice (wild-type and *il6*$^{-/-}$) to other scientific groups within the University of Cambridge. We have given mice away (wild-type and *gcn2*$^{-/-}$) to our collaborator at Sheffield under an MTA.

3.4 Please **LIST** the techniques and/or animal models you have used on your licence (include GA models). Later on you will be asked about the 3Rs achievements – please **do not** detail your 3Rs achievements in this box.

1. Baseline *gcn2*$^{-/-}$, *il6*$^{-/-}$ and *gcn2*$^{-/-}$,il6$^{-/-}$ models (protocol 7)
2. Wild-type and *gcn2*$^{-/-}$ with chronic LPS for induction of pulmonary vascular disease (protocol 8)
3. Wild-type and *il6*$^{-/-}$ with mitomycin-C for induction of pulmonary vascular disease (protocol 8)

3.5 How successful have these techniques and/or models been?

All models have shown a pulmonary vascular phenotype.
Reversal of the baseline *gcn2*$^{-/-}$, *gcn2*$^{-/-}$ with chronic LPS, and wild-type with mitomycin-C was achieved by genetic ablation of *il6*.

3.6 What limitations, if any, have you encountered? Were you able to overcome these limitations and, if so, how?

All non SARS-CoV-2 mouse work was halted temporarily during the lockdown phases of the pandemic. The main researcher had a spouse with a terminal illness during these period and so most of the work planned was carried out by a collaborator instead.

Some of the work has been a little delayed due to these factors. However, the majority of the work is completed and is in submission at PNAS. The PhD student (Max Schwiening) has successfully completed his studies and is now a postdoctoral researcher at AstraZeneca, having won 2 awards for his work done on this licence.

3.7 Have you attempted or been able to refine any of your techniques or models? **NO**

Please provide a list of techniques or models you have been able to refine and the refinements you have made. Please indicate if you have published or disseminated this information during the tenure of this licence and attach a copy of the publication.

3.8 Do you use systems to monitor the welfare of your experimental animals (e.g., scoring sheet)? **YES**

If not, why?

Animals are weighed and inspected thrice a week in the chronic dosing protocols. The use of MCMS also allows us to track trends in weight.

If you do use a welfare monitoring system, what do you use? Did you refine or modify your system during the tenure of your licence and what impact did this have?

The use of MCMS allows us to input weight and health observations and to track trends in weight.

No modifications.

3.9 Were the end points detailed in your project licence appropriate? **YES**
Have you needed to modify them during the tenure of your licence? **NO**

Please explain.

The 2 models we have used were of the severity expected (moderate) and the majority of mice (99.78%) survived to the endpoints (heart catheterisation under anaesthesia as a terminal procedure).

3.10 Please review the non-schedule 1 methods of killing authorised on the protocols on your licence. In the box below, list each method and beside each clearly indicate which you have used? Examples of non-schedule 1 methods include: exsanguination, decapitation and perfusion fixation.

The non-schedule 1 methods of killing authorised are
a) Perfusion-fixation (not used)
b) Exsanguination and completed by a Schedule 1 method (used).

Were these killing methods the most refined? **YES**
Were they the most appropriate for your experimental data? **YES**

If you have answered NO to either of the above questions please explain. If you were able to refine the way your animals were killed please explain how this was achieved.

Not applicable.

3.11 Have you received awards or funding specifically targeted at the 3Rs? **NO**

If so what was the source of your funding, when did you receive it and what did the funding allow you to achieve?

Not applicable.

The AWERB 3Rs committee is particularly interested in this section of your review because information you provided here could be of interest and use to other researchers at the University.

Information that you have included in this section will be included in the UBS 3Rs Search Tool unless you complete the box below. UBS will only add information to the UBS 3Rs Search Tool section on the UBS website that University staff can log into using their Raven Access. You should however disseminate all 3Rs finding more widely.

I do not agree to information provided in this section to be added to the UBS 3Rs Search Tool for the following reason(s).

Not applicable.

4. Scientific Progress and Benefit Achievements:

4.1 Taking into consideration that your licence may not be due to expire, has your research progressed as you expected? **YES**

4.2 When your licence is due to expire will you have completed the programme of work described in this licence? **NO**

4.3 Regardless of whether you managed to complete your programme of work, what have you achieved/discovered during the time since this licence was granted? (Please copy your key scientific questions/objectives which appear at the beginning of Part D, or in the Action Plan, of your project licence into the box below and against each explain what you have achieved/discovered).

Action plan objectives (from SJM PP5789170)

1. **Characterise models of pulmonary hypertension that involve defects of the "integrated stress response" (ISR).**
   We have fulfilled this aim, by creating and characterising 2 mouse models, one with genetic loss of GCN2 and the other with a toxic insult (mitomycin-C) that involves loss of GCN2. Both lead to a pulmonary vascular phenotype, physiologically (proven on heart catheterisation) and histologically.

2. **Modify the ISR genetically to modulate pulmonary hypertensive phenotypes.**
   We have fulfilled this aim, by showing that genetic ablation of *il6* rescues the pulmonary vascular phenotype in both of the mouse models above.

3. **Modify the ISR pharmacologically to modulate pulmonary hypertensive phenotypes.**
   We have started on this aim – we can now show that increasing the ISR is protective against inflammation in the acute setting. We have also shown the converse – that blocking the ISR is detrimental, also in the acute setting.

   Our forthcoming project will focus exactly on this – utilising the information from our acute studies to create dosing regiments to stimulate the ISR and/or to block the IL-6 pathway in order to ameliorate the pulmonary hypertensive phenotypes.

   We also have collaborators who are prepared to share their ISR activators for us to test in our mice models.

4.4 For key scientific questions/objectives you could not complete either fully or partly, please include an explanation as to why you were not able to achieve what you set out to achieve when the licence was granted (reasons could for example include lack of funding, the model took longer than expected to develop, another group published data which meant continuing the work would equate to repeating the work, etc.).

We were significantly delayed by the SARS-CoV-2 pandemic, during which all laboratory and mouse work not directly pertaining to SARS-CoV-2 was temporarily ceased in the University, and resources were diverted to SARS-CoV-2 research.

The main researcher undertaking the mouse work was also ‘shielding’ as her husband was undergoing chemotherapy. She then subsequently took a significant leave of absence when her husband’s condition progressed and became terminal.

4.5 In the expected benefits section of your licence you described the benefits you believed would be likely to arise from your programme of work. Please explain what benefits have actually been achieved

and, if appropriate, explain how these differ from what you anticipated you could achieve when you wrote the licence.

We have done the following: a) Created 2 new mouse models of GCN2-associated pulmonary vascular disease, where none previously exist, which allow for testing of therapeutic options. b) Identified that one of the key drivers for GCN2-associated pulmonary vascular disease is inflammatory in nature and that targeting IL-6 works to prevent the development of GCN2-associated pulmonary vascular disease. c) Specifically identified IL-6 as a clinical biomarker in mutation-driven pulmonary hypertension in humans, which surpasses NT-proBNP.

4.6 Specifically, if you were unable to address any or even all your key scientific questions/objectives what impact did this have on the benefits arising from your work?

This has delayed the path to a translational trial resulting from this work.

4.7 Were you able to identify/develop/implement any new scientific or technical developments during the tenure of this licence? **YES**

If so, what were these and what were/was the benefit(s)?

We have established that anti-inflammatory and/or stimulation of the integrated stress response is a possible option to target GCN2-associated pulmonary vascular disease/pulmonary veno-occlusive disease. There are no therapeutics currently available for patients with PVOD and so this represents a significant step forward.

4.8 Using the headings below please provide a list of publications and meetings attended where **research arising from work undertaken under this licence** has been disseminated.

Please highlight in bold the publications which contain and meetings at which 3Rs achievements were disseminated.

Please only include publications arising from research undertaken under the authority of this licence. If you have papers in press please include these.

Publications in scientific journals: See below, in submission at PNAS.
Publications pending, or under review: Interleukin-6 is critical in the development of gcn2-mutation associated pulmonary vascular disease in mice https://www.biorxiv.org/content/10.1101/2025.04.14.648729v1
Presentations at scientific meetings: 1. Mutations in GCN2 cause pulmonary vascular disease via dysfunctional inflammatory pathways. Max Schwiening *et al.* British Thoracic Society 2023. 2. Unpicking the Effects of General Control Non-depressible 2 (GCN2) Deficiency in Pulmonary Veno-Occlusive Disease. Max Schwiening *et al.* European Respiratory Journal 2023; 62: Suppl. 67, OA3155. **Winner of ERS Young Investigator Award**. 3. Towards a murine model of pulmonary veno-occlusive disease. Max Schwiening *et al.* British Thoracic Society 2022. **Winner of BTS abstract prize**.

Have you remembered to highlight in bold the publications which contain and meetings at which 3Rs achievements were disseminated?

4.9 Have you engaged with the general public by giving interviews about your work on radio, television, at a public meeting or debate or in a newspaper article? **YES**

If you answered NO: why?

If you answered YES: how, where (i.e., what medium did you use) and when did you engage with the public, what sort of information did you disseminate and what was the outcome?

A recorded interview was done at CIMR by Dr Soon for the Academy of Medical Sciences aimed at encouraging young people to join the sciences.

Selected public engagement work by Marciniak

2021

- Talk (online): Cambridge Festival, "The Grander View, how modern microscopy illuminates a rare disease" 27 March 2021
- Talk (online): Sutton Trust (a charity which aims to increase university applications from individuals from disadvantaged backgrounds), August 2020
- Video podcast for "The Naked Scientists" on Lung Physiology, 5 March 2021 [https://youtu.be/eV2WnUF0CAg]

2022

- ISAC (Inspiring Scientists at CIMR): lab experience and mentoring event for less-advantaged Y12 pupils
- School Talk, Longstands Academy, St Neots 27th June 2022
-

2023

- School Talk, Longstands Academy, St Neots 23rd May 2023
- RareFest 2022, provided hands-on microscopy activities for children, 26 November 2022
- ISAC (Inspiring Scientists at CIMR): lab experience and mentoring event for less-advantaged Y12 pupils
- Hosted and spoke for 'Melanin Medics, a non-profit charitable organisation for the present and future African and Caribbean doctors and clinical researchers, February and September 2023

2024

- School Talk, St Neots Academy College 12th Jan 2024
- School Talks, Wells Academy, Nottingham, 15th Jan and June 2024
- ISAC (Inspiring Scientists at CIMR): lab experience and mentoring event for less-advantaged Y12 pupils, Feb 2024
- Hosted and spoke for 'Melanin Medics, a non-profit charitable organisation for the present and future African and Caribbean doctors and clinical researchers Aug 2024

2025

- Melanin Medics, August 2025

5. Project Licence management information.

5.1 Amendments (if applicable):
Provide dates and a summary of all amendments made to this licence (i.e., what each amendment requested and why it was required) since the licence was granted (or if appropriate since the licence was last reviewed).
Please read questions 2.11 and 2.12 – for amendments that apply to this question including those that apply to either 2.11 or 2.12, please provide the date of the amendment here and, if relevant, a note that further information can be found in Section 2.11 or 2.12.

No amendments have been requested.

5.2 Project licence Standard Condition 18 Reports (if applicable):
Provide dates and copies of all Condition 18 reports sent to the Home Office since the licence was granted (or if appropriate since the licence was last reviewed). Copies of any reports should be attached/supplied as separate documents please.

Both SC18 reports are attached with this.

5.3 Non-Compliance (if applicable):
Provide the date, a summary of the nature of any non-compliance and the outcome (or if appropriate since the licence was last reviewed).

Not applicable.

5.4 Compliance Assurance Meetings [CAMs] or Root Cause Analysis (RCA) meetings (if applicable):
Provide the date, a summary of the nature of any incident and the outcome, if known (or if appropriate since the licence was last reviewed).

Not applicable.

5.5 Have there been any other issues that have affected the management of this licence that you feel should be taken into consideration, (e.g., equipment failure, absence of key staff, change in the health status of the animal unit etc.)?

Not applicable.

6 Retrospective Review:

Project Licence Holder check list:

AWERB requires the following documents	Documents sent to AWERB
Completed copy of this form	x
Copies of Condition 18 reports (if applicable)	x
Copies of 3Rs publications (if applicable)	

For UBS office use only:

	Date
1. Retrospective review form sent to project licence holder	10/06/2025
2. Retrospective review form received by the Project Support Team	
3a. Project Support Team meeting	
3b. 3Rs committee	
3c. AWERB Standing Committee	
4. Process completed	