The University of Manchester

ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 17 October 2024

Present: [redacted]

Apologies: [redacted]

1. **Minutes**

Agreed: That the minutes of the meeting held on 19 September 2024 were approved.

2. **Applications for New Project Licences**

2.1. [redacted], How Immune Responses Regulate Metastasis & The Evaluation of Immunotherapies.

Considered: A completed AWERB form, PPL application and presentation.

Interviewed: [redacted]

Committee discussion:

- The Chair invited members to raise areas of concern or clarification which were subsequently discussed with the applicant.
- The committee decided which of the pre-submitted questions or points of clarification could be addressed in the feedback letter and which they wished to discuss in person and in depth with the applicant.

Discussed with applicant:

- The committee thanked the applicant for a clear presentation.
- The committee questioned the applicant on the possible use of analgesics in the animals they are inducing tumours in. The applicant explained that analgesics work by being anti-inflammatory, which is the system they are looking at, therefore their use could impact the results. The committee understood this explanation but would still like the applicant to discuss with the NVS the possibility of finding a painkiller they could use that would not affect the immune response.
- The committee noted that in response to the question in the Category A form 'Please explain if you will publish negative findings and make the raw data open access' the applicant had stated "no as results may be commercially sensitive". AWERB is committed to ensuring that researchers publish outputs from their work, including so-called 'negative' findings, i.e. where hypotheses have not been proven, where there is scientific value. The committee queried with the applicant if they were not planning on publishing all findings, or did they mean they would not make the raw data open access. The applicant outlined the obstacles that researchers face when trying to publish work that has not supported a hypothesis, including how this may impact on future funding decision. In addition, in this specific case, the researcher expressed concerns about how the research field would interpret any 'negative' results for compounds that have not produced the desired result, in that results can be 'negative' for several reasons and just because it has not worked in a specific experiment in a specific lab does not mean it would not work elsewhere. The applicant also explained that some of the compounds are proprietary, and the publishing of any results requires consultation with the company. A discussion took place on the differences between producing a publication and making the data open access, the latter of which does not require a 'story' as would a publication. The committee understood the points raised by the applicant and agreed to discuss the matter further at an 'away day' to ensure they fully understand all the competing issues. They would, however, ask that the researcher of this current licence to be open minded about sharing data and to commit to publishing all findings negative or otherwise where possible. Improving cultures of, and approaches to, publishing negative findings, is an ongoing endeavour across the research community, and it has particular ethical consequence for animal research.
- The committee were aware that the applicant had previously carried out research with zebrafish and queried with the applicant why they had moved to models in mice, given the advice to use the least sentient animal where possible. The researcher explained that one reason for the change was practical in that many reagents developed for human targets would likely work in mice, but not fish. Another view expressed by the applicant was that there is a reluctance of the research committee to believe the results from zebrafish models, which can also impact on funding.

Revisions: It was explained to the applicant that the committee had provided comments to the Secretariat prior to the meeting and while some would

be discussed in the meeting, the list below includes all the comments whether they were raised in the meeting or not.

- The title is a bit long and complex - does 'of novel therapeutics' need repeating? is there a way to use less technical language for 'melanoma metastasis'?
- As discussed in the meeting, please speak with [redacted] regarding the mandatory/optional steps in the licence to ensure that they allow you to carry out the experiments as you intend to.
- Please discuss with the Named Veterinary Surgeon the possibility of using analgesics. The committee understand the limitations which you explained during the meeting and the need to balance the scientific outcomes with the potential use of analgesics.
- The committee discussed with you the possibility of publishing work that has not supported your hypothesis. The committee are sympathetic to the issues of researchers publishing so-called 'negative' results, especially when the results may be commercially sensitive. However, AWERB ask that you commit to making the data available where possible so that the wider research community can learn from the outcome(s). The committee understand that whilst a compound may not have worked in one lab this does not automatically mean it isn't viable, but they do want to reduce the risk of studies being replicated unnecessarily.
- It would be beneficial to add some information, as you explained in the meeting, on where the therapeutics will be sourced from and information on if the relevant dosing and tolerability studies have been carried out, or will be carried out in tumour free animals, prior to being given to those animals who have tumours.
- Protocol 3 – please check that the interventions planned to be carried out are possible as the licence is now written. The licence states 500 mice which seems to be a large number if no experimental manipulations are taking place.
- Page 28 - [redacted] The breeding protocol is a mild one and so the committee presumes that you are not able to breed the NSG mice themselves. Please can you clarify this.
- Page 30 – please seek advice on if more information is need on the surgery to implant "tumour pieces". e.g. will analgesia be given?
- Page 31 - Have you considered using an alternative to oral gavage?
- Page 32 - is weighing animals once a week enough if they have active tumours? could they lose weight rapidly in this model and therefore surpass an HEP?
- Page 32 – the body condition score is not defined, but the committee expect it is Ullman-Cullere 1999. Please include more information to confirm which scale is being used.
- Page 33 - For non-invasive imaging, there are 6 options (not all explained). Which are the most/least refined? If there is some variation can there be some justification of not using the most refined method?
- Page 36 – The end point for step 6 is if weight loss of 20% occurs but in the control and monitoring section no weighing of mice is outlined. This should be included.

- Page 40 – the discussion of the statistical design mentions excluding animals which bite or scratch the tumour. I couldn't see any mention of this under adverse effects/HEP – please seek advice from the Named Persons on including this.
- A number of comments were made regarding your Non-Technical Summary which are listed below. Please update your NTS based on the comments and send it to the following lay members for their review ([redacted])
    - Page 2 of 53 - can melanoma, metastasis and gene expression plasticity be expressed in non-technical language or concisely defined in non-technical language?
    - Page 3 of 53 – "expand the pipeline" please can you clarify the meaning for non-experts.
    - Page 4 - I suspect lay people will struggle with the concept of de-differentiation. Can you make it more accessible to a lay reader?
    - Page 5 - Fourth line: Can you explain why 'moderate pain would only be experienced for hours to days'? Why cannot pain be avoided altogether? Also, consider using the phrase 'genetically altered' mice rather than 'recombinant mice' so the lay reader might understand more easily. Perhaps also replace the last seven lines in the first paragraph, beginning with "For imaging embryos" simply with: No adverse effects are associated with the gm modified line of mice we are using called iDct-GFP.
    - Page 5 of 53 - The wording does not make it entirely clear what proportion of animals will experience what - do all non-breeding animals experience moderate? What proportion of the 3800 would this be?
    - Page 7 - Please consider adding the information on gavage and injections into the 'what will be done to the animals' section on page 4. Rather than list them again on page 7, you could say that they are routine procedures and will be performed by highly trained staff. Though minor procedures, I would also add tissue sampling (ear biopsy and mouth swabbing) to the "what will be done to the animals" section on page 4.
    - Page 8 of 53 - published guidance - have PREPARE been consulted and will ARRIVE be utilised? Can we state this commitment for clarity in line with UoM policy.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

2.2. [redacted], Long-Term Effects of Developmental Hypoxia on the Maternal & Fetal Cardiovascular System

Considered: A completed AWERB form, PPL application and presentation.

Interviewed: [redacted]

Committee discussion:

- The Chair invited members to raise areas of concern or clarification which were subsequently discussed with the applicant.
- The committee decided which of the pre-submitted questions or points of clarification could be addressed in the feedback letter and

which they wished to discuss in person and in depth with the applicant.
- The committee were informed why the applicant was submitting two related licences and the rationale for the research not being in one licence.

Discussed with applicant:
- AWERB thanked the applicant for the very clear presentation.
- AWERB queried how many animals will be pregnant at the same time. The researcher explained that the pregnancies are staggered so that the pups are born in batches.
- Where possible AWERB would like group housing of animals and asked the researcher about the procedure under the licences. The researcher explained that animals are kept on their own during pregnancy so that weight and water intake can be monitored, but once the first pup is weaned they are group housed.
- Where possible AWERB would encourage researchers to use both sexes to avoid surplus animals. The researcher explained that previous studies have shown the males are more affected by hypoxia, therefore the licence will use only male animals.

Revisions: It was explained to the applicant that the committee had provided comments to the Secretariat prior to the meeting and while some would be discussed in the meeting, the list below includes all the comments whether they were raised in the meeting or not.
- As mentioned during the meeting, please check that the number of animals to be used on the licence is correct. 250 mice are listed in Protocol 1 so the committee queried if this would be enough.
- Please include details on the use of only male animals, given you explained in the meeting that the males are more affected by hypoxia.
- A number of comments were made regarding your Non-Technical Summary which are listed below. Please update your NTS based on the comments and send it to the following lay members for their review ([redacted])
    - Page 2 of 46 and the Title - hypoxia - is there an alternative non-technical way to express this that the non-expert would recognise and understand? Such as 'lack of oxygen' used on p.3?
    - Page 3 - minor point but "in adulthood" is a bit odd given mother is probably/usually adult? Could you clarify this by removing or changing to 'later in life'?
    - Page 3 - In the output section, it may be beneficial to mention that the work will also contribute to an understanding of disease mechanisms.
    - Page 3 - In the section "Maximizing Output," it could be beneficial to mention engagement with industry, as it was referenced in a previous section. Also, have you considered adding 'media engagement' to the section on maximising the outputs of this work? [redacted] would be more than happy to discuss this with you, as Animal Research Communications Lead.
    - Page 4 - Under “what will be done to the animals” you might add that the mice will have minor procedures including ear biopsy,

blood sampling, hair sampling and mouth swabbing. Also consider saying in this section that you will induce cardiac arrhythmias in the mice.
    - It may be beneficial in the refinement section to state that the animals will be given analgesia when needed.
    - Page 4 of 46 – please use a non-technical alternative for "ion channels" or briefly explained what they are.
    - Page 5 of 46 – please use a non-technical alternative for "intrauterine" or briefly explain the term.
    - Page 5 - 68% mild and 32% moderate; it is difficult to map these numbers to the description of impacts in NTS; where is the moderate severity - surgery? on p.7 it is suggested hypoxia causes moderate but in the description it here it suggests hypoxia causes little distress?
    - Page 5 - Could you re-order the bullet point list of non-animal alternatives so it follows the same sequence as the explanation in the next question? This would be easier to follow.
    - Page 8 – the use of PREPARE is good to see flagged; perhaps also flag the use of ARRIVE for publications.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

2.3. [redacted], Long-Term Effects of In Vitro Fertilisation on the Maternal & Fetal Cardiovascular System

Considered: A completed AWERB form, PPL application and presentation.

Interviewed: [redacted]

Committee discussion:
- The Chair invited members to raise areas of concern or clarification which were subsequently discussed with the applicant.
- The committee decided which of the pre-submitted questions or points of clarification could be addressed in the feedback letter and which they wished to discuss in person and in depth with the applicant.

Discussed with applicant:
- Where possible AWERB would encourage researchers to use both sexes to avoid surplus animals. The researcher explained that they have not documented sex differences in IVF studies, therefore, therefore the licence will use both sexes.

Revisions: It was explained to the applicant that the committee had provided comments to the Secretariat prior to the meeting and while some would be discussed in the meeting, the list below includes all the comments whether they were raised in the meeting or not.
- As mentioned during the meeting, please check that the number of animals to be used on the licence is correct. 250 mice are listed in Protocol 1 so the committee queried if this would be enough. Protocol 3 lists only 9 mice, which as you explained in the meeting is likely to be a typographical error.
- Please include details on the use of animals from both sexes.

- A number of comments were made regarding your Non-Technical Summary which are listed below. Please update your NTS based on the comments and send it to the following lay members for their review [redacted]
    - Page 3 - In the section "Why is it important to undertake this work?", it would be beneficial to provide more detail on the specific types of cardiovascular diseases associated with IVF procedures. Including prevalence rates or relevant statistics would help strengthen the case.
    - Page 3 - In the output section, it may be beneficial to mention that the work will also contribute to an understanding of disease mechanisms.
    - Page 3 - In the section "Maximizing Output," it could be beneficial to mention engagement with industry, as it was referenced in a previous section. Also, have you considered adding 'media engagement' to the section on maximising the outputs of this work? [redacted] would be more than happy to discuss this with you as Animal Research Communications Lead.
    - Page 4 - Under “what will be done to the animals” you might add that the mice will have minor procedures including ear biopsy, blood sampling, hair sampling and mouth swabbing and the fish will have minor procedures including biopsy of caudal fin, swab of surface mucus, fin clipping of larva, and micro-abrasion of embryos. Also consider saying in this section that you will induce cardiac arrhythmias in the mice.
    - Page 5 - Can you explain briefly in the NTS what happens to the fish during the swimming tests and hypoxia challenges? The bracketed word 'recovery' doesn't seem to make sense - perhaps say instead the fish fully recover afterwards?
    - It may be beneficial in the refinement section to state that the animals will be given analgesia when needed.
    - Page 6 - Could you re-order the bullet point list of non-animal alternatives so it follows the same sequence as the explanation in the next question? This would be easier to follow.
    - Page 6 - "Exceptions - There are some questions within our study that could be answered using cell lines. For example, if we identify drug targets, we could genetically manipulate cell lines (e.g. knockout experiments) to confirm that our targets are important. " - but will you? Perhaps you could clarify where possible if you will.
    - Page 9 - the use of PREPARE is good to see flagged; perhaps also flag the use of ARRIVE for publications.

Outcome: The study was given provisional approval based on the applicant making the changes/clarifications listed above to the satisfaction of the Chair/AWERB.

3. Report on licences processed from 04/09/2024 to 01/10/2024

The following amendments were approved by the executive committee.

3.1. Amendments to Project Licences

[redacted], Genes and Essential Nutrient Influences on Behaviour
[redacted], The Impact of Integrin a1b1 Signalling on Polycystic Kidney Disease
[redacted], Understanding Serosal Repair & Internal Scarring

3.2. Amendments to Project Licence [redacted], Breeding and Maintenance of Genetically Altered Rodents

[redacted] Generation of SP-LgBit driver Mouse Line Using CRISPR

4. Update on applications outstanding from previous meetings and upcoming Project Licence applications

4.1. The committee were provided with a document showing the status of applications considered previously and those pencilled in for future meetings. A verbal update was given as some licences stated as being submitted to ASRU had now been granted.

5. NVS report

5.1. The Chair asked if the NVS could explain at an away day how the system works for when they are called out to the animal facility.

6. 3Rs AWERB subgroup report

6.1. The Chair noted that FRAME (Fund for the Replacement of Animals in Medical Experiments) which was mentioned in a previous meeting has updated its name to Replacing Animal Research.

6.2. It was noted that a researcher who had recently submitted their retrospective review discussed how it was getting easier to publish negative results. The Chair of the 3Rs subgroup observed that it is likely to be field specific.

6.3. The reproducibility crisis was mentioned which is where it is difficult or impossible to reproduce the results of many studies.

6.4. A discussion took place on the term 'negative results'. It was noted that this is the term generally used, however it was also raised that 'negative results' are experiments where the hypothesis was not proven, and can still be beneficial in informing the research community.

6.5. The following paper was provided 'Doing good science is hard': retraction of high-profile reproducibility study prompts soul-searching (nature.com)

7. Any other business

7.1. 3Rs symposium

The Chair of the 3Rs subgroup encouraged the scientists on AWERB to speak with their labs and wider colleagues about submitting posters for the symposium which is taking place on 14 November 2024

7.2. ASC (Animals in Science Committee) AWERB Hub Workshop

The Chair and Compliance and Licensing Manager attended the AWERB Hub Workshop on 16 October which was held online.
The papers are available for AWERB members at the following link: [redacted]
The North West AWERB Hub meeting is taking place next week.

7.3. Practical work on frogs

The Chair provided an update on the actions he had taken since the away day on 8 October 2024 where the committee discussed an application submitted under Category C for a practical in the Physiology Research Skills Module for 2nd year undergraduate students. The practical aims to demonstrate to students how nerve stimulation in vertebrates results in muscle contraction and uses the intact sciatic nerve and gastrocnemius (calf) muscle of frogs.

7.4. Zoom/Teams

AWERB meetings will be moving back to Zoom. Teams was trialled however the functionality required by AWERB is not available in Teams.
Notifications in Teams cannot be turned off which distracts committee members and applicants. People on the call can be made 'attendees' by the meeting organiser, however those required to give a presentation need to be made 'presenter' when they join which allows them to be notified of people in the lobby and given the same capabilities as the meeting organiser, such as muting people, starting or stopping recordings, changing meeting options and having access to the meeting chat even after they have left the meeting.
Given the sensitive and confidential nature of the meeting these capabilities should not be available to everyone.
The Secretary and Chair will monitor if the functionality of Teams changes which may make it possible to move from Zoom to Teams at a future date.

The next meeting will be on 14 November 2024 at 10am-12.30pm.

Dates of meetings for the 2024/2025 academic year are:
12 December 2024
30 January 2025
27 February 2025
27 March 2025
24 April 2025
29 May 2025
26 June 2025
31 July 2025
August break

Dates of meetings for the 2025/2026 academic year are:
25 September 2025
23 October 2025
20 November 2025
18 December 2025