

ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 20 October 2022

Present:

[REDACTED]

Apologies:

[REDACTED]

1. Minutes

Agreed: That the minutes of the meeting held on 22 September 2022 were approved.

2. Applications for New Project Licences

2.1. [REDACTED], Mechanisms of Fungal Infection & Drug Resistance

Considered: A completed AWERB form, PPL application, and minutes from Local Management Committee Meeting

Interviewed: [REDACTED]

- Discussed:*
- [REDACTED]
 - Agricultural companies are developing similar drugs that are more quickly approved for spraying on crops so there is an urgent need to understand drug resistance.
 - Clarity was sought about the monitoring periods.

- Revisions:*
- Page 23 - protocol 1 uses adult mice. The licence elsewhere refers to using mice as young as 6 weeks – please can you clarify if these are considered adult.
 - Page 23 - Protocol 1 Step 1 - administration of antibiotics in the drinking water - no adverse effects noted here. Are these palatable? Have there ever been instances of dehydration or weight loss?
 - Page 26 – the scoring sheet for monitoring animals doesn't say what the consequences are for reaching a certain score so additional information is needed in the licence. You explained in the meeting that based on historic data, you have determined that if any mouse hits a threshold of 3 in any category they will be humanely killed immediately. If a mouse reaches a score of 2 in a single category and have a score of 2 for more than 36 hours they would be humanely killed at that point. If they score 2 in two or more conditions they would be humanely killed immediately.
 - You explained in the meeting that animals are monitored every 12 hours until they show signs of infection and then the time would be reduced to 10 hours. Please include this information in the licence.
 - Protocols – the gaps between successive steps, i.e. repeat injections, could be tightened up and explained throughout.
 - Page 27 - What is doxycycline being used for?
 - 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

- The NTS is generally clear for a lay reader, however there are still some places where it is too technical and it would benefit from being shortened.
- Page 3 - Under 'Who or what will benefit from these outputs, and how?', there is a bit of repetition of the info in the "why is it important to undertake this work? section which could be edited out I think.
- Page 3 - This is clear and as a lay reader I do understand it but one might consider removing part of it as it is additional technical detail that may not be entirely required to follow the point -"the triazoles (itraconazole, voriconazole, posaconazole and isavuconazole) the echinocandins (caspofungin, micafungin and anidulafungin) and the poorly tolerated polyene, amphotericin B. Voriconazole is the first line therapeutic for the treatment of all forms of aspergillosis."
- Page 3 - "it has now reached a point where there is consensus that this is an imminent global public health crisis that requires immediate action" could be more concise for instance "this now poses an imminent global public health crisis that requires immediate action"
- Page 4 - Who or what will benefit? - Please define 'CPA'.
- Page 4 - "The rationale for using mice of this age has not been well established..." - I appreciate the transparency here but it

may confuse the lay reader who may ask - why not? The second half of this sentence seems to imply a rationale even if it is not well established it appears clear? I am not sure we need this caveat and it could be considered for removal?

- Page 5 - Typically what will be done - Line 5 - Too much detail, in the sentence 'in studies measuring fungal burden. Suggest omit after 'set time point'.
- Page 5 - what is meant by severely affected? This may need qualifying for understanding.
- Page 5 - " will immediately culled." consider changing to "will be immediately humanely killed".
- Page 8 - I found some of the text under Which animal models and methods will you use rather difficult to understand. Could phrases like neurophysiological sensitivity, fungal burden analysis and competitive fitness analysis could be explained in lay terms?
- Page 8 - I read that Galleria melonella is in fact the Greater Wax moth caterpillar I think? Perhaps it would be helpful to say this?
- Page 9 - Can you explain what the difference is between fungal burden experiments and virulence studies. Why are fungal burden experiments more humane? Why the need to do both?
- Page 25 - Can you add the use of non-aversive handling techniques to minimise the level and duration of stress to the refinement section of the NTS? Also that mice are placed in cages with sufficient environmental enrichment to minimise stress and are handled as infrequently as possible?
- Page 32 - Please remove the reference to the supplier [REDACTED].

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], Regulation of Basement Membrane Function in Health & Disease

Considered: A completed AWERB form, PPL application, and minutes from Local Management Committee Meeting

Interviewed: [REDACTED]

- Discussed:*
- Creatinine levels may change in mice and the proposed spot urine collection approach may not give the required measures. There is no perfect marker in terms of urinary excretion. The project is most interested in albumin levels using creatinine as an indicator of concentration. A discussion of how acceptable this method is took place.
 - Zebrafish are an attractive model for the kidney as there is a single kidney filter and two tubes that are drainage tubes.
 - Some treatments going to be tested are already used in the clinic but more understanding is needed about why they are working. The researchers intend to improve treatments by understanding better how they work. A range of interventions will be chemicals and gene therapies.

- Appropriate competencies will be checked regarding the fish undergoing imaging and anaesthesia.

Revisions:

- As per the discussion at the meeting, protocols 1 and 2 should be split into two with each becoming a mandatory step so it is easier to understand what an animal will experience. In addition, the gaps between steps need to be explained in more detail i.e. between successive steps.
- As discussed in the meeting, a statement explaining that albumin/creatinine measures are not perfect but are the best available at this time should be added to the licence.
- Page 20 – please explain what hemizygous males are.
- Page 22 - "animals will be maintained by methods appropriate to their genetic alteration until they reach a maximum of 8 months of age". Elsewhere (page 20) it suggests maintenance of some genotypes to 12 months. Please can you clarify this and update the licence so that it is consistent.
- Page 23 – please include answers to questions about randomisation and group sizes.
- Page 33 - "failure to urinate will results in mouse being killed by Schedule 1 method". Is this going to be hard to measure? How would you notice if a mouse in its home cage wasn't urinating?
- Page 51 - Here gavage tubes are said to be sugar coated but this is not included elsewhere. Please update this where appropriate.
- Page 52 - Under "What are the likely steps...." what volume appears to be unnecessary.
- Page 71 – please obtain advice from the Named Persons on the use of males only. There is a move towards using both sexes.
- Page 72 – Typo “adta” should be data.
- Page 72 – please explain how you are going to measure proteinuria.
- Page 72 - Under “Why scientifically do the animals need to suffer to this degree” please remove the reference to mice as protocol 5 relates to Zebrafish.
- Page 74 – please include details on which kinds of genes will be edited.
- Page 75 - Typo "teh" should be the.
- Page 77 - Under step 2, where are Casper fish females from? Please seek advice from the Named Persons if this needs to be separate from the Protocol.
- Page 81 – please explain how the fish will express fluorescent proteins.
- Page 82 – “For each model and/or method, what is the scientific need for the expected clinical signs?” Typo “gins” should be “signs”.
- 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 contact information]

- General NTS. I am not sure it's particularly clear what will be done to the mice and what will be done to the fish in the NTS. So for example, you could say in terms that both mice and fish have injections and that analgesia is also administered to fish. I feel the public won't be aware that fish can feel stress or pain so it's good to spell that out.
- Page 2 - penultimate line on page - what's a "basement membrane" - this is the NTS, so technical language needs explaining, especially if the term is a key one such as this.
- Page 2 – the section on importance and section on benefits repeat information such as "Chronic kidney disease (CKD) is a huge public health concern, affecting more than 10% of the global population and substantially increasing their all-cause mortality." I suggest these be edited so that information is not repeated and each section only presents what is required.
- Page 4 - Minor typo: 'published in a ranger'. Should be range
- Page 4 - Please define peptides, MRI, and Intravital imaging.
- Page 4 - if it might be helpful to add in the NTS - maybe under what is to be done to the animals - a line on Alport syndrome, and why this condition in particular is being studied and that it presents more in males. Though Alport syndrome is listed as a key word on P3 of the NTS, there is no mention of it in the body of the NTS.
- Page 4 - I would like to see more on what will be done to the animals in terms of what stresses them. A mention of oral gavage, repeat blood sampling/injections, ear biopsy, hair sampling, mouth swabbing, blood pressure monitoring via the carotid. And that the mice during urine collections will experience mild stress due to isolation away from home cage and cage mates.
- Page 5 - Please define HEK cells.
- Page 5 - "Why do you need to use" paragraph: "In addition to standard cell culture, we have also developed organoids cultures as a more complex culture system a closer mimic of environment in vivo." It feels as if something has got lost in this sentence.
- Page 5 - "Which non-animal alternatives" For the non-technical reader this sentence needs clarifying throughout.
- Page 6 – Please ensure the numbers in the licence and on the Cat A form match.
- Page 7 - I thought you might expand a bit on refinement procedures. For example, cage enrichment having adequate size entrance holes to ensure there is no risk of skin catching/rubbing following surgery seems like a good thing to document. Maybe also frequent monitoring during post-operative recovery period.
- Page 7 - perhaps remove "with glomeruli and branching collecting ducts" as it is not easily understood by a lay reader and not required to get the point of the sentence.

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. Mid-term review and amendment of Project Licences requiring full committee review

3.1. [REDACTED], Mechanisms of diabetes-associated heart disease

Considered: A completed mid-term review form, amendment summary sheet and PPL with amendments highlighted.

Interviewed: [REDACTED]

Discussed:

- The rationale for adding rats to the Project Licence was discussed including that not all work done in mice will be done in rats.
- The work done in the rats will complement that done in mice.
- The NTS will change following approval of the amendment and the new NTS should be made available on the website.

Revisions: None.

Outcome: The amendment was given approval.

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. Two people who were pencilled in for the November 2022 meeting have not progressed with their applications enough so will be moved to later meetings. There is no animal welfare issues due to this delay.

5. NACWO and Directors report

5.1. A typographical error (complaint not compliant) was noted in the ASRU audit report otherwise no questions were made on the report submitted.

6. NVS report

6.1. No comments were made on the report submitted.

7. Standard Conditions 18s and non-compliances

7.1. The committee were provided with a table of reports submitted to ASRU along with the reports for each incident. Three incidents were discussed. [REDACTED] stepped out of the meeting for the two issues that he is involved in investigating.

[REDACTED]

Animals went past humane points due to the study moving from one licence to another which had different humane end points. An investigation is taking place by the BSF compliance committee which will feed back to ASRU. Rather than relying on a printed version of the licence it should be accessed via ASPeL so that it is clear what is allowable under the licence and the information is always up to date.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

8. 3Rs AWERB subgroup report

- 8.1. No comments were made on the minutes and reviews submitted.

9. ASRU audit report and response

- 9.1. The BSF were complemented on the audit report. Follow-up reports were requested so that AWERB could have assurance that matters were being addressed.
- 9.2. Both major risks identified by the audit were administrative in nature and not serious or impactful on animal care. The major risks have been addressed and the BSF can provide the committee with a written report on how the matters were dealt with. There were 11 minor risks. There are meetings scheduled within the BSF to address them and AWERB will receive a report that has to be endorsed by ASRU.

10. Any other business

10.1. Update on away day discussion on digital cage solutions

■ and ■ reported that the digital cage solutions that were discussed at the away day do not do what was originally thought. The request for infrastructure funding to support buying any of these cages has been put on hold.

10.2. Talk by Professor John Ioannidis from Stanford

A meeting is taking place on 26 October 2022 about the 3Rs strategy and will be followed by a talk from Professor John Ioannidis from Stanford.

The next meeting will be on 17 November 2022 at 10am-12.30pm.

Dates of meetings for the 2022/2023 academic year are:

22 September 2022
20 October 2022
17 November 2022
15 December 2022
26 January 2023
23 February 2023
23 March 2023
27 April 2023
25 May 2023
22 June 2023
20 July 2023
August break