MANCHESTER 1824
The University of Manchester

ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 27 April 2023

Present: [redacted]

Apologies: [redacted]

In attendance: [redacted]

1. **Minutes**

Agreed: That the minutes of the meeting held on 23 March 2023 were approved.

2. **Applications for New Project Licences**

2.1. [redacted], Zebrafish Models to Investigate Disease Processes Associated With Brain Haemorrhage

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

Interviewed: [redacted]

Panel discussion:

- The statistician had not seen the application prior to the papers being circulated but is happy with the planned experiments and to sign it off.

Discussed with applicant:

- A discussion took place about the timescale in clinical cases compared to those in the planned experiments. The zebrafish haemorrhage in the morning and there is a 2-3 hour window afterwards before they are treated.
- The researcher discussed why genotyping using both skin swabs and fin clipping is being asked for, explaining that less DNA is sometimes obtained from skin swabs which means that the genotyping doesn't work, but with fin clipping there is more of the sample so genotyping results are guaranteed.
- The researcher explained that the variation in swim time for the vehicle treated intracerebral haemorrhage naïve fish is because this test is carried out when the fish are young. Younger fish can be less active so some won't be swimming in the 10 minutes during which they are observed, but the swim test needs to be done early on because of when the intracerebral haemorrhage is induced. The committee note that this means that a higher experimental number of animals is needed but understand the reasons behind this.
- More detail was given by the researcher on how the maximal suffering from a stroke is controlled and the traffic light system that will be developed and used.
- The benefits of using zebrafish models prior to mammalian models was discussed.
- The applicant explained that for the older fish when they are held in gel a water flow over the gills is required and provided.
- The types of drugs being tested was discussed and if any of them would be neuroprotective.
- The method of humanely killing the fish was explained to the committee.

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.

- Page 5 – please seek advice from the BSF if a severity/harm statement is needed regarding the repeated cycles of retro-orbital injections.
- Page 6 – please clarify if any of the mutant animals exhibit abnormal behaviour (should they then be S1 as per recovery from anaesthesia - this could impact adversely on experimental outcomes).
- Page 7 – please seek advice from the BSF if you need to include a report on pre-regulated 'head count'.
- Page 12 - Have you got proof-of-concept? Might a conservative peer-reviewer knock back a paper because it's using something from the wrong species, thereby wasting the work you've done and the fish you've used?
- Page 23 - What are the indicators of if a fish has an infection?
- Page 28 – at present it reads that there is no option to repeat sampling for genotyping. Is this correct, and if so, will these cause

issues if the genotyping from the swab doesn't work, i.e. does the licence as currently stated stop you from obtaining a fin sample from the same animal?

- Page 29 – Step 4 for killing is optional which appears as an inconsistency for an end of a protocol. Please seek advice from the BSF if more detail should be added to explain that the animals may be used for other protocols.
- Page 29 - Protocol 2, Step 3 is mandatory and says "maintenance to the age of 18 months" implies all fish will be kept to this point, modify to "maintenance **up** to the age of 18 months".
- Page 30 - How likely are the animals to become egg-bound and how dangerous is that for them? Can this be added to the NTS?
- Page 35 - For administration of drugs - does AA need to be added for when given in water - currently stated as AB and AC?
- Page 36 and 47 - For some of the behavioural monitoring is this not done using AA (although maybe it is obvious it will be AA)
- Page 40 - In response to "how will you minimise variables to ensure reproducibility" more detail is required.
- 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 – if possible in the Home Office online system, please explain some of these terms for lay readers, e.g. cerebrovascular integrity, translational.
    - Page 3 - Benefits Section - What outputs do you think you will see at the end of this project? "We will generate new zebrafish models" needs briefly explaining in this NTS.
    - Page 4 - "Who or what will benefit" paragraph mentions "stroke" - should this term appear earlier along with brain bleeds/haemorrhages?
    - Page 4 - Project harms section - "pre-protected" needs explaining for lay readers.
    - Page 4 - How will you look to maximise the outputs of this work? Can you also include mention of how you will maximise publication of 'negative' results from the work.
    - Page 4 - The projected benefits are all plausible, but maybe it'd be good to be a little more circumspect about the way they're articulated. If such certainty about the benefits were justified, research wouldn't be: after all, uncertainty is in the nature of research.
    - Page 5 - Typically, what will be done to an animal used in your project? – this section is too detailed for an NTS and would benefit from being reduced.
    - Page 5 - Some mention of how brain injury is induced in the pre-protected stage in this section would be helpful. There is mention of studying fish post-injury but it would help to have

insight into how the injury is induced (even though it's not covered by this licence).

- Page 5 - This section still has singly housed for maximum of 5 days which contrasts to the protocol where it is after 3 days you co-house; please update the PPL so that it is consistent.
- Page 5 – In the last paragraph can you include some details of what the drug will be in the water.
- Page 5 - Perhaps you can use the term 'humanely killed' as opposed to just killed? Though the description makes the case implicitly, it may be wise to be explicit that the technique is humane.
- Page 5 - Could you say more about the immobilisation of the fish? Doesn't that imply reduced water-flow, and therefore a risk of asphyxiation?
- Page 6 - What are the expected impacts and/or adverse effects for the animals during your project? – phenotype & genotype need explaining, as does NVS.
- Page 6 – Can you include an estimate of how many fish are likely to be killed due to showing abnormal behaviour or signs of infection or is this information not known at present?
- Page 6 - What are the expected severities and the proportion of animals in each category (per animal type)? Can you give a brief explanation of what "sub-threshold means".
- Page 7 - How have you estimated the numbers of animals you will use? – the sentence starting "In reality however…" could benefit from rewording and explaining what a clutch is.
- Page 8 - What steps did you take during the experimental design phase to reduce the number of animals being used in this project? – this section needs some more detail for a lay reader, including an explanation of "heterozygous mutant incrosses".

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], Understanding Serosal Repair & Internal Scarring

Considered: A completed AWERB form and PPL application

Interviewed: [redacted]

Panel discussion:

- The statistician still needs to sign off on this work after the applicant has made the suggested changes.

Discussed with applicant:

- The control arms of the study. Clarification is needed in the application.
- The adverse events as currently listed do not adequately reflect the procedures and should be amended.

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.

- There are some minor typographical errors in the application which need correction.
- AWERB understand that further input is required from the statistician before they can approve the application.
- Both males and females will be used in these experiments. Please include some information on whether you expect sex differences in incidence or severity of adhesions as in the experience of one scientific AWERB member inflammatory responses to LPS can vary by sex.
- Please seek advice from the BSF if a breeding protocol is needed for some of the transgenic mice.
- Page 21 - Protocol 1 Step 1 (and also Protocol 2, Step 1) - there should be a maximal number of tamoxifen doses and minimal interval between dosing included.
- Page 22 and 33 – please include details on what the control arm is if inflammation induction is the mandatory step.
- Page 23 - LASA guidelines are no longer used. Please seek advice from the BSF on which guidelines are to be used instead.
- Page 23 - What are the humane endpoints for this step? Please seek advice from the BSF and NVS on if allowing a period of 24 hours to pass is acceptable or if animals showing the signs of ill health that you describe should be humanely killed before 24 hours have passed.
- Page 30 - Why are you proposing this severity category? The sentence starting "We will perform two surgical procedures" requires rewording for clarity.
- Page 34 - Why is AB needed for i.p. injections - can these not be done under AA?
- Page 34 - Are examples of inflammation inducing agents needed?
- A number of comments were made regarding your Non-Technical Summary which are listed below. However it was praised for its clarity for a lay audience: 'this is something of a model of how to describe a complex piece of research to a non-technical reader'.
- Please update your NTS based on the comments and send it to the following lay members for their review [redacted]
    - This is very clearly written
    - Please add to the NTS that the mice will receive up to a maximum of 7 IP injections of inflammation-inducing agents, that some animals will receive oral gavage and how the animals will be humanely killed.
    - Page 4 - Typically, what will be done to an animal used in your project? Instead of 'bugs' could you use 'bacteria' or 'micro-organisms'?
    - Page 5 - What are the expected impacts and/or adverse effects for the animals during your project? The NTS and this section in particular gives the impression that no adverse effects happen however as you explained in the meeting this is not the case as

you monitor the animals closely. Please can you reword this section to reflect this.
- Page 6 - What steps did you take during the experimental design phase to reduce the number of animals being used in this project? Please explain the terms wild-type, heterogenous and homogeneous.
- Page 7 "To induce gene expression in GA"; please can you explain these terms for lay readers. Otherwise this is something of a model of how to describe a complex piece of research to a non-technical reader.

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 reviews of Project Licences requiring full committee review

3.1. [redacted], Development and validation of animal models for neurodevelopmental disorders

Considered: A completed mid-term review form.

Interviewed: [redacted]

Panel discussion:
- Mid-term reviews are usually considered at the 3Rs subgroup unless they contain severe protocols. While this licence does not have any severe protocols it was felt that due to the contents of a recent amendment the mid-term review should be considered at the full committee.

Discussed with applicant:
- The researchers have not observed abdominal contractions following i.p. injection to the pregnant dams which the amendment added as a potential adverse effect.
- The researchers are looking to tissue share with another group.
- A discussion took place on if the pregnant female or the pups are the experiment unit.
- Translation into humans was discussed.

Outcome: AWERB support continued work on this licence.

4. Report on licences processed from 06/03/2023 to 12/04/2023

The following amendments were approved by the executive committee.

4.1. Amendments to Project Licences

[redacted], Immune & Inflammatory Mechanisms in Cerebrovascular Disease
[redacted], Understanding Endogenous Protective Mechanisms in Osteoarthritis; Towards a New Approach For Disease Management
[redacted], Genes and Essential Nutrient Influences on Behaviour
[redacted], Peripheral Nerve Regeneration
[redacted], Immunopathology of Experimental Blood-Stage Malaria

[redacted], Imaging & Radiation Treatment of Cancer

4.2. Applications for additional availability for new or current project licences

[redacted]: Gut Pathogen & Microbiota Effects on Host Health *(additional availability for use of Germ Free facility only)*

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

5.1. The committee were provided with a document showing the status of applications considered previously and those pencilled in for future meetings.

6. NVS report

6.1. A discussion took place on the Analgesia Guidance document that was submitted as part of the December 2022 NVS report. Given some drugs are used off-label the point was raised on if AWERB need a statement about this. A further discussion will take place at the next meeting.

7. 3Rs AWERB subgroup report

7.1. No comments were made on the minutes and paperwork from the most recent 3Rs AWERB subgroup meeting.

8. Podcast

8.1. A 'ground rules' document was circulated outlining how the proposed Podcast would work. AWERB members present approved the ground rules and agreed to proceed with the Podcast.

The next meeting will be on 25 May 2023 at 10am-12.30pm.

Dates of meetings for the 2022/2023 academic year are:
22 June 2023
20 July 2023
August break

Dates of meetings for the 2023/2024 academic year are:
21 September 2023
19 October 2023
16 November 2023
14 December 2023
25 January 2024
22 February 2024
21 March 2024
25 April 2024

23 May 2024
20 June 2024
25 July 2024
August break