ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 16 November 2023

Present: [redacted]

Apologies: [redacted]

In attendance: [redacted]

1. Minutes

Agreed: That the minutes of the meeting held on 19 October 2023 were approved.

2. Applications for New Project Licences

2.1. [redacted], Central Nervous System Development: From Cellular Decisions to Developmental Disorders

Considered: A completed AWERB form and PPL application.

Interviewed: [redacted]

Committee discussion:

- All three projects still require statistical sign off.

Discussed with applicant:

- The applicant was asked why 13% was chosen as the HEP for weight loss. She explained that this was a compromise as it was felt a 5th of body weight would be too much.
- The applicant was asked if 72 hour housing if the zebrafish would be sufficient. She explained that this would be achievable after fin clipping. DNA can be process quickly and PCR can be conducted in house and she has a technician who does this routinely.

- Protocol 1 proposes the breeding of 3,000 mice, whereas Protocol 2 uses only 300. The applicant was asked to explain the difference. She explained that the majority of the research will use transgenic reporters in Protocol 1. Experimental work will be conducted in vitro, taking cells from embryos from Protocol 1 . Protocol 2 aims to manipulate genes in the embryo. This is not done very often.

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.

- Title is quite academic - is there a way to convey purpose in simple lay language? Maybe something like "Investigating developmental stem cell decision making to better understand congenital eye disorders'?
- Pg 14 - The aims are a bit confusing - there are three knowledge gaps, three aims that don't directly track to the gaps, a hypothesis that is focused on the third gap, then general molecular
- pg 14 - “We will define : (2) sounds a bit generic - which genes, or how are they related to each other?
- Pg 15 - The objectives don't directly track to the above gaps or aims. There are two additional aims, and objective 1 has more aims and two sets of experiments and objective two has phases, and two more phases for objective 3.
- Pg 16 - There might be unexpected defects that could arise - avoid raising the possibility of "anything could happen"
- Pg 42 - You propose to use 300 mice for protocol 2 and 3250 for protocol 1 - so will the majority of mice produced in protocol not have injections to induce phenotype?
- 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]
    - Pg 3 - Style is quite scientific and heavy going for the lay reader but just about makes sense. Terms such as microphthalmia and coloboma require explanation or simpler language.
    - Pg 4 - “Evolutionarily conserved” is a bit technical could you just say similar?
    - Pg 5 - Are most of the zebrafish being experimented on larval stages and not under licence? Could specify this in the 'what will be done' if true.
    - Pg 6 - re-capitulate - does this require explaining or simplifying for the lay reader? Is it another word for model?
    - Pg 6 - The nature of the expected mild severity was not quite clear, that will be experienced by the 10% mice that receive a transgene inducing agent. Is it like the zebrafish- some visual impairment? Could this be clarified?
    - Pg 6 - "We propose to use genetically altered mice and we expect the phenotype of these animals to be sub-threshold (90%), the mice that receive a transgene inducing agent will experience mild severity (10%)." It would be helpful to unpack

the reference to phenotype here (and to do the same with knockout in the previous paragraph.
    - Pg 6 - in vitro organoid models - this needs explaining (unless I've missed something earlier in the NTS)
    - Pg 7 - Reduction section - nb the guidelines/instructions do say "Do not mention POWER calculations here. If relevant, there will be an opportunity to provide these details elsewhere."

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 Disease Mechanisms in Frontotemporal Dementia & Motor Neuron Disease.

Considered: A completed AWERB form and PPL application.

Interviewed: [redacted]

Committee discussion:

- Disease scoring system is clearly laid out. The maximum score is 12. The protocol allowed the animals to reach 10. Want to explore if 10 hit was severe. This had been discussed with the applicant extensively during pre-AWERB.
- How will the intercranial injections be performed. The size of brain in mouse pups, the needle will not hit a defined structure in the brain as it would in an adult.

Discussed with applicant:

- The HEP scoring system was developed by colleagues at [redacted] and is useful to ensure time is spent assessing the animal. The applicant plans to refine the scoring system during the pilot studies. It was agreed that the applicant would work with the NACWO and NVS to adjust to less extreme end points, where possible.
- The applicant confirmed that reference to sutures can be removed.
- The applicant explained the ICV injection model. The skin on mice pups is translucent. Landmarks can be seen on the skull. The skull is thin. Thy will inject dye to confirm they are hitting the correct position. This should be made clear in the application. The BSF will work with the applicant on cadavers and scale up. There is an experienced licence holder who can be worked with to refine the technique in advance of progressing to live animals. Could also visit collaborators in USA.
- AWERB agreed that the applicant should be Invited back to AWERB in early 2025 to provide an update on the project.

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 says adenovirus, should be AAV?
- Pg 9 – maybe do not state 'failure' quite so directly
- pg 12-13 - The phenotype correlation between mouse and human for the different pathogenic changes, is complex and a bit confusing.

- Pg 26 - To be discussed with NACWOs, but perhaps if an animal is found moribund that should also be added to HEPs (standalone from the DSS)?
- Pg 32 & p53 - Should the timeframe for the HEPs be amended here to say ">8h" or is "<8h" correct?
- Pg 32 - Presumably the ICV injections are under anaesthesia? Is there any more detail about this specific procedure that you could add, e.g. anaesthetic code
- Pg 41 - Why would an animal have a suture prior to imaging in step 8 protocol 2?
- Exp Design - No power calculations are provided. We appreciate that there is close involvement of a biostatisticiain, but how were animal numbers for the licence generated?
- Pg 53 - weight loss of >20% is stated twice in the HEP section
- Distress Scoring SystemClearly there are several ways of reaching a score of "10" but some combinations appear as more "severe" than "moderate". Please discuss.
- 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]
    - Pg 3 - second paragraph: understudied - better (and less "theatrical") as under studied?
    - Pg 4 & following - The project harms section is a bit complex to read, it might be worth re-reading it and redrafting it with the non-technical reader in mind. In particular the "Typically, what will be done" paragraphs use too many acronyms and references to tests and procedures which are not explained: these paragraphs feel as if they haven't been drafted with the non-technical reader in mind. By contrast, the "what are the expected impacts" paragraph
    - Pg 4-5: ‘We also considered using C. elegans worms, Drosophila or Zebrafish for this work, however, immune function differs substantially between mammals and other species. Furthermore, we require the use of in vivo imaging techniques which are not available in lesser species such as flies, worms and fish e.g. PET or MRI scans.' This statement can be covered under question 'Why can’t you use animals that are less sentient?' p.8 . So does not need repeating here.
    - Pg 4 – the first sentence of project harms isn’t clear. Justifying use of GAA mice as you need to perform behavioural tests?
    - Pg 5 – there are a few typos "are get", "be non-recovery anaesthesia", blood will "be collected on more than 6 occassions" should this be "will be collected on NO more than 6 occassions"?
    - Pg 5 ‘(e.g. Rota-rod, open-field test, Y-maze etc.) usually at 2 or 3 timepoints' probably too much information and could be removed from NTS.

- Pg 7 - Reduction section: I quote from the instructions/guidelines: Do not mention POWER calculations here. If relevant, there will be an opportunity to provide these details elsewhere.

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], Immunoregulation in Helminth Infection

Considered: A completed AWERB form and PPL application.

Interviewed: [redacted]

Discussed with applicant:

- HEP could be included on the cage card. However AWERB was satisfied that % weight loss was factored on the study plan and staff were very experienced with these models..
- AWERB liked the additional slides that all presenters included that showed changes made after pre-AWERB. it was good to see how ethical considerations has been thought through.

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.

- Overall, the PPL reads very clearly to the lay reader. However, the Title could be revised to make clearer sense to the lay reader; for instance, "Immunoregulation in parasitic worm infection' or perhaps 'Investigating how the immune system responds to parasitic worm infection' Removing technical words will help lay reader to see the meaning of the project in the title.
- Pg 24 - Please clarify the difference between the two different weight loss HEPs - do you mean if one animals loses 15% in 24 hours that it will be culled (aka the speed of the weight loss indicates the HEP?)
- Pg 34 - it is worth making clear that cage cards state and make very clear which organisms the mice have been infected with, as the humane endpoints for each infectious agent are different (e.g. 15% weight loss or 18% weight loss), so that BSF staff will know what is normal/expected for each infection anc can properly care for the mice.
- Pg 103 - Should be clear that both mice and rats will be used to passage parasites (as Step 1 is non optional) - therefore the sentence "Specifically, mice will be administered" should be edited to include rats, or another statement should be made about similar regimens for rats. Similarly, throughout other steps and study design sections, wording should be careful to include both mice and rats - looks like much is copied from other protocols and has no mention of rats. Do you expect the same level of severity in rats, and the same end points?
- 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]

[redacted]

- Project harms section: "The availability of an extensive array of tools including antibodies recombinant proteins etc. together with gene targeted and transgenic mice make the mouse an unrivalled system to study. For studies of the microbiota the availability of germ free and gnotobiotic animals provide unique and powerful approaches to be employed." In the context of very clear introductory material, the sentences quoted here seem to lose focus on the non-technical reader, using a number of technical terms which need explaining (and even after some years on AWERB I need help with "gnotobiootic"!)
- Which non-animal alternatives section: much as the previous comment. It would be worth redrafting parts of this section for the nontechnical reader who won't understand in vitro or organoid
- Reduction What steps did you take...? The non-technical reader may not need the detail in statistical methods you provide here - something much simpler would suffice and be better understood.

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 02/10/2023 to 31/10/2023

The following amendments were approved by the executive committee.

3.1. Amendments to Project Licences

[redacted], Immune and inflammatory mechanisms in cerebrovascular disease
[redacted], Early safety assessment, investigatory and efficacy studies
[redacted], Regulation of basement membrane function in health and disease.

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

[redacted] Creation of INPP5b flx/flx Mouse Line Using CRISPR
[redacted] Creation of Col4a2 Mouse Line Using CRISPR

3.3. Applications for Category C work

[redacted] Characterisation of Cardiac Cellular & Vascular Function in Coronary Artery Disease (CAVCAD)

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.

5. Standard Conditions 18s and non-compliances

5.1. The committee were provided with a table of reports submitted to ASRU along with the reports for each incident.

5.2. The Compliance and Licensing Manager reported that all Standard Condition 18s were submitted to ASRU in a timely manner as stipulated by ASRU. Due to a period of absence of an inspector in ASRU from 14th February, which the BSF was not informed about until mid-summer, no feedback has been received February. We are now receiving Standard Condition 18 feedback from ASRU and have received assurances from the Inspectorate that all Standard Condition 18s are now being reviewed. However we will not hear back unless ASRU has concerns. This is the new practice. We as establishment are taking responsibility for checking that everything is working appropriately.
On the back of this change, the senior named staff are reviewing SC18s on a 6 monthly basis to see if they can identify any patterns/elements that require greater oversight and interrogation or if there is any learning. They will feed back to regulated community and to AWERB when the meetings happen.
Had a couple escalated to possible non-compliances, currently with ASRU.

6. Any other business

6.1. The NVS was in the unit on Tuesday and no concerns were picked up.

6.2. The AAALAC accreditation visit is taking place on 23 and 24 November.

6.3. The Registrar gave a huge thank you to everyone on the committee. He commented that it is important to the University and his role as Establishment Licence Holder. He thinks the committee has been refined and is effective, and members do an amazing job.

The next meeting will be on 14 December 2023at 10am-12.30pm.

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

Dates of meetings for the 2024/2025 academic year are:
19 September 2024
17 October 2024
14 November 2024
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