MANCHESTER 1824
The University of Manchester

ANIMAL WELFARE AND ETHICAL REVIEW BODY

Minutes of the meeting held on 22 June 2023

Present: [redacted]

Apologies: [redacted]

In attendance: [redacted]

1. **Minutes**

Agreed: That the minutes of the meeting held on 25 May 2023 were approved.

2. **Applications for New Project Licences**

2.1. [redacted], Biology of Brain Tumours

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

Interviewed: [redacted]

Committee discussion:

- The tables are missing from the application. This happens when the applications are downloaded in pdf format from the HO system.
- AWERB want to know more from the applicant about the severity banding and what steps are in place to prevent the severe severity band being reached.

Discussed with applicant:

- The severity limit being severe rather than moderate. The committee were reassured through the monitoring schedule and use of the seven point scale that the applicant will be able to pick-up quickly when animals reach stage 4 and are required to be humanely killed.
- The committee understood the explanations from the applicant and the NVS why analgesia cannot always be given. It was explained to the applicant that is may be useful if they included some brief information on this on Page 5 with regards to the sentence 'providing analgesia, when required and when possible without negatively impacting the course of the experiment'.
- The committee raised if oral gavage can be avoided and heard from the applicant the situations where this might be possible and AWERB understood the reasons why certain drugs cannot be administered this way.

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 a few typographical errors in the application which would benefit from being corrected.
- The title is good, clear and gives a good lay sense of the topic. You might wish to consider adding a simple verb such as Investigating the.." or "Understanding the…"
- Page 9, please remove the submitted manuscript from the list as it is not needed here and it will not be findable in the future. If the paper is accepted during revision of the PPL then you can add in PMID.
- Page 19, How often will non-invasive imaging occur? Is there a limit on frequency?
- Page 24/101, Protocol 1 - would benefit from listing mouse strains (or at least some GAA strains) as it stands there is an effective carte blanche here; is n = 1000 adequate?
- Page 25, 15 months sounds fairly old, is there any specific reason for this?
- Page 31/101, Protocol 2 – we would advise that you perhaps include other methods of recombination rather than just Cre/LoxP)
- Page 31, 33, 34, 55, 57, 58, 65, 79, 81, 82 – the tables are not shown in the pdf document that the committee received. Please provide these and ensure they are present on submission to the Home Office. It was explained to the committee that these tables have been assessed in the pre-AWERB meeting and no concerns were raised.
- Page 31, Step 1 (and in other protocols). The title of this step "modulation" is vague – please consider rewording.
- Page 32/101, are all mice reconstituted? Is this bone marrow transplant, does this need to be explained for the non-specialist?
- Page 32/101, the radiation dose isn't lethal if reconstituted there should this be reworded i.e. avoid saying following lethal radiation?
- Page 33/101, Is step 3 really mild? I.e. no adverse effects expected and by definition only mild and transient discomfort. You state multiple cranial injections in a few days with GA.

- Page 34, are adverse effects for tamoxifen needed? This is the same for the other protocols.
- Page 38/101, step 7 - should there be a separate protocol for the GA models so that it becomes clearer how many animals will have the windows and injections intracranially vs the GAAs who won't have the injections? - this is the only compulsory step but what % of animals will be surgical vs GAA only?
- Page 42/101, it is stated that the animal will be anaesthetised daily for 14 days – please provide clarification on what duration, how is it delivered, how long will it last for, etc. This point also relates to Protocol 3.
- Page 34 and in all Protocols. For non-invasive imaging please include if there is a recovery time between imaging sessions where you state the animals may be imaged up to twice a day for no more than 7 consecutive days.
- Page 43/101, anaesthesia daily for 5 days for extended 4 hr imaging modalities is starting to look rather more of a severe banding than moderate; please ensure you include a clear work up of the percentage of animals going through each step so that the overall experience can be judged more carefully. As it stands it is difficult to get a good feel for the overall animal experience (despite the flow diagrams). This point also relates to Protocol 3.
- Page 45, For termination, do you need cardiac perfusions of substances (e.g. saline/fixative) if taking tissue for e.g. immuno? This is the same for the other protocols.
- Page 45, what will happen to the tissues post-mortem? I didn't get a feel for the kind of experiments that will be performed to answer the tumour environment / ECM objectives.
- Page 58, step 4, are there really no more than mild and transient adverse effects from i/c etc injections. Should this not be considering what the compound might do in the longer term rather than just the short term (same in protocol 2).
- Protocols 3 and 4 - The same comments with regards the steps in Protocol 2 also apply here and require clarification and revision.
- 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]
    - Overall the NTS was considered well written for lay members but there are some areas that would benefit from being more concise with less technical detail included.
    - I did not get a sense from the NTS what sex the mice will be; whether one or both sexes would be used. Might this matter? Reading further into the application it seems both sexes will be used. Would this be useful to state in the public facing NTS (as sex difference is beginning to be widely understood in public imagination as shaping likelihood of developing illnesses in humans)?

- Page 3, all outputs are required to be open access under University of Manchester policy. Please remove the words 'when possible' from the section on maximising out of the work to reflect this policy.
- Page 4, Please seek advice from the BSF if an upper limit should be used instead of the stated > 60 days.
- Page 4 of 101, 'Explain why you are using these types of animals and your choice of life stages' - the answer to this question is appropriate for lay reader but could be broken up from the current one long sentence with many clauses to a couple of shorter sentences. This would aid readability.
- Page 4 of 101, some terms such as piloerection and ataxia may not be comprehensible to the lay reader and if possible alternative non-technical terms would be preferable if possible; however the general sense that these are signs of suffering is clear.
- Page 6 of 101, I am not sure what 'co-culture' means in reference to in vitro systems - is there a way to make this understandable for a non-expert concisely without technical terms? Or would in vitro suffice (still a technical term but I think we assume generally a lay reader looking at NTS statements would know what this is).
- Page 6, it may be helpful to explain what "perturbation" in this context is.
- Page 6 of 101, in vivo - you might wish to add '(in animal)' or words to that effect although as mentioned this term may be assumed to be widely understood in the NTS context.
- Page 6 – final paragraph, Final paragraph: You use the statistical term "power" here - it's not a transparent term for the lay reader so please consider revising.
- Page 7 of 101, 'replete immune system' - I was not sure what replete means in relation to the immune system and how this answered the question as to why lower sentience species were unsuitable.
- Page 7 – the section on refinement could contain a line on prophylactic antibiotics are given before and after irradiation; under what will be done, the creation of cranial windows could be included as they will have a fairly major impact on the animal; also under refinement you could add that the behavioural tests are non-invasive and designed not to cause pain or stress, which could impact positively on results but also welfare.
- Page 8 of 101, 'How will you stay informed…' this is a minor point for your consideration - the current answer leads with being on a mailing list which can be read as a very passive way to receive information and following compulsory institutional rules; I am not sure this presents what you mean or how you will work in its most positive light. You might wish to gently rephrase this around more positive actions; stressing your teams commitment to staying informed by regularly surveying the literature; working with the institution to improve practice and such (newsletters are fine of course but balanced with action). It

would be good to get the sense that your project is not just passively receiving and implementing welfare improvements but working to contribute to the identification, development and implementation of better welfare when possible (as I am sure you will be this is just a suggestion for presentation).

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], Molecular Basis of Infection-Induced Sickness Behaviour

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

Interviewed: [redacted]

Committee discussion:

- The licence is based on one that the researcher was granted at another institution.
- The applicant was very much engaged with the BSF staff during the drafting of the licence and took on board any suggested changes.

Discussed with applicant:

- Maintenance of mice up to 15 months (Page 58 of 63, Protocol 3, Step 3 – Maintenance). The applicant explained that they are not interested in mice of this age and 15 months is recommended by ASRU. AWERB discussed that animals have previously not been kept to this age on a breeding protocol and that the BSF will report back to AWERB on any adverse effects or observations should animals be kept to this age. The applicant explained that you don't usually tend to use animals that are older than 3 months.
- The applicant clarified that they do not routinely keep animals in the dark for 80 days consecutively and that it is usually for 10 days. The applicant was asked to amend Page 4 of 63 to explain this.
- The scientists on the committee discussed with the applicant the use of a high fat diet and in their experience the animals tend to enjoy this rather than not liking it. The applicant was asked to include some details on page 42 of 63 on how long will diet be given for and when they will provide this, as they explained in the meeting.
- The applicant explained that they have previously seen on average retrospectively a weight loss of 10%, therefore the requested limit of 15% provides some leeway. The AWERB committee supported this percentage but asked that the animals be closely monitored as the researcher has not carried out the model at this facility so the average is based on that from another facility.

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 a few minor typographical errors which would benefit from being corrected.
- Overall it was difficult to get a feel for the typical and extreme animal experience which is needed to make a risk/harm/benefit assessment.

- Humane end points. Page 29 lists >15% will lead to euthanasia but on page 35 you state that "If mice show an immediate loss of 20% of starting body weight, then the animals will be euthanized immediately". Please seek advice from the BSF if the percentage should be the same.
- Please can you ensure that the number of animals requested in the licence matches that listed in the Cat A form.
- Would it be beneficial to swap Protocols 2 and 3? This would mean that Protocols 1 and 2 would be 'tool generation' and Protocol 3 would be the experimental protocol.
- Page 13 of 63, please can you explain what CXCL10 means.
- Page 14 of 63, "What new knowledge do you hope to discover that will address a gap in fundamental scientific knowledge or meet a clinical need?" It may be beneficial to start with the short term gains rather than jump in with a new chemotherapeutic regimens, which is a much longer-term aim.
- Page 15 of 63, Objective 1, is obtaining blood stream tryanosomes a difficult/unprecedented thing? It seems like more of a minor technical aim otherwise although this may just how it is worded.
- Page 20 of 63, is the s/c route any less effective than IP or ICV route for infection? Do you need all three routes of administration? Is one route more likely than the others? Do they result in different outcomes?
- Page 21 of 63, the volumes of blood limits is not stated (assumed negligible if films and microsampling) and needs clarification.
- Page 21 of 63, Protocol 1 - two blood sampling steps are listed. Perhaps step 4 could be made the terminal procedure (and then I don't think it matters if they are exanguinated as that is part of method of killing).
- Page 22 of 63, Protocol 1 step 2 and step 4 have the same title. Please revise.
- Page 23 of 63, please seek advice on if a specific Schedule 1 method needs to be listed.
- Page 23 of 63, In Protocol 1 under general adverse effects it's said that mice may lose their appetite - does this lead to weight loss and if so should this be monitored? This point isn't directly addressed as an adverse effect/HEP in the Step itself.
- Page 25 of 63, Protocol details - can you give a sense of the information that will be generated i.e. examine the parasite for what? What kind of knowledge will you gain?
- Protocol 2, Step 2 (infection monitoring) and Step 3 (blood sampling by venepuncture) appear to be essentially the same although one is mandatory and one is optional. What is the frequency and limits on Step3? These limits in Step 3 should take into account Step 2.
- Page 27 of 63, Please consider if a more robust reason for using harmful GAA is needed.
- Page 27 of 63, would it be better to separate out step 1 to two steps to differentiate between no risk sc/ip infection using AA and the AB intracerebral injections (or at least provide a clear outline if one step on how many will go down each route) - making the animal experience easier to follow.

- Page 27 of 63, Protocol 2 - weight loss doesn’t appear as a standalone HEP.
- Page 29 of 63, There are some discrepancies - in the text it states 'flaccid paralysis of both hind limbs' is grade 4 and animals will be kept for up to 72h and if no improvement they will be culled. However, the table above states 'flaccid paralysis of both hind limbs' is grade 5 and animals will be euthanised immediately. Please ensure consistency.
- Page 32 of 63, does the table need to be clearer regarding frequency of injections - e.g. 21 applications at X frequency (row 1 as an example).
- Page 32 of 63, it is also not clear if there will be a combination of treatments so the number of interventions could be staggeringly high. Please clarify this.
- Page 32 of 63, 30 craniotomies is a considerable amount and the table gives more an impression of severe suffering potential and needs to provide much more clarity on routes, frequencies, volumes and limits.
- Page 33 of 63, Under "How will you monitor for….." should not all adverse effects be tested for not just some.
- Page 34 of 63, Step 5 - IP tamoxifen for 10 doses seems excessive and is likely to have major adverse effects. It is listed as a maximum of 10 doses but please include what it is likely to be.
- Page 36 of 63, Should not all optimum doses be determined not just some?
- Page 37 of 63, Under "What are the likely adverse effects…" Should not all effects be tested for in pilot studies? Please state that you will monitor for adverse events.
- Page 37 of 63, you mention rat here but only mice are listed on page 26 as being used in Protocol 2. Please clarify if you are using rats as well as mice.
- Page 39 of 63, Please repeat the adverse effects in each step rather than cross refer to earlier steps.
- Page 42 of 63, If you're monitoring mice for depression phenotypes when they are in complete darkness for up to 80 days, won't the mere fact that they are in darkness for so long in itself be associated with depression?
- Page 43 of 63, Protocol 2, Step 10 - the title of this step might be better as "modulation of light/dark cycles". Also, mention in HEP that mice may be rehoused in pairs - should this be limited to females?
- Page 45 of 63, Please explain the choices of radiation doses as others have stated it is lethal at 11Gy whole body.
- Page 52 of 63, The statistician requested that the first paragraph be reworded as follows: ‘For instance, if it was required that a real increase in % MAdCAM1+ CD21/CD35+ cells of 4 percentage points (ie 10% to 14%) should be detected with 80% probability then 7 animals per group would be required, assuming that a 1-sided 5% test was used. This assumes that the standard deviation is 2.6%. The naïve control level of % MAdCAM1+ CD21/CD35+ cells is 10%, so this change represents an increase of around 40% over control.’

- 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]
    - Thank you for a well-written NTS. Perfect for a lay reader! A general comment that (in replacement) the illustrative example of how a culture model of the impact of trypanosome infection on the blood brain barrier showed different results from a mouse model is really helpful to the lay reader.
    - Page 2 of 63, a good deal of the answer to 'aim' is more appropriate as an answer to 'importance'. For example "It affects some of the poorest regions in sub-Saharan Africa. The disease can be fatal to both humans and domestic animals causing significant social and economic hardship. Although trypanosomiasis has been recognised for centuries, many aspects regarding how the parasite interacts with its host remain unknown" all reads as justification for the work as opposed to a concise explanation of the aim of the project.
    - Page 4 of 63, "What are the expected impacts". Please replace the word ‘lifeless’ with moribund.
    - Page 4 of 63, immunomodulatory' is a technical term - you may wish to offer a concise definition or use a non-technical alternative in the NTS for the non-expert reader.
    - Page 4 of 63, ‘may decline considerably becoming lifeless' - would ''may decline considerably' suffice? It is slightly unclear what lifeless means here given they response is to humanely kill the animal. Presumably decline considerably and become - extremely lethargic, comatose?
    - Page 4 of 63, You don't need to repeat "typically", but it would be appropriate to give some indication of the numbers/percentages undergoing surgery and irradiation rather than simply saying "Additionally" and "In some instances"
    - Page 4 of 63, "rear events" should be "rare events"
    - Page 5 of 63, first paragraph: does checking every 3 days equal "close monitoring"?
    - Page 5 of 63, ‘Human tissue obtained from stem cells generated on a dish' - thank you for defining organoids so clearly.
    - Page 5 of 63, 'transendothelial migration ' – please clarify this for a lay reader, as with tissue colonisation and dissemination.
    - Page 5 of 63, 'and as long as it helps us address specific experimental questions' - you may wish to delete this clause. I think we can assume you would only use such methods if they were useful for your aims?
    - Page 5 of 63, Replacement paragraph: "using a culture...mouse…. model" is not clear to the NTS reader - perhaps better to say something like "in the culture flask … in live mice."

- Page 6 of 63, Reduction section: "factorial design" doesn't mean anything to the NTS reader - either explain it, or (maybe better), omit it.
- Page 6 of 63, Please consider rewording “When appropriate, we will conduct pilot experiments to determine appropriate sample size and power calculations” as it reads as though power calculations are not done for every experiment.
- Page 6 of 63, "breeding colonies will be maintained at a minimum size". Please can this be elaborated on.
- Page 6 of 63, The answer to "what steps did you take during experimental design process to reduce numbers of animals used" doesn't really address the question. Please update the response.
- Page 7 of 63, ‘Where possible, we will use chemical compounds such as Busulfan as alternative, less aggressive, methods to irradiation in order to manipulate the immune system.' - this sentence felt a little out of place - why is it relevant to the question asked? Can this method (Buslfan) only be used with mice? Or is it trying to say that when it comes to irradiation alternative methods that are less aggressive will be used? Perhaps something could be added to clarify as the sentence did not seem to follow clearly from the prior sentence in answering the question. Bulsfan is mentioned in greater detail and more clearly at the bottom of p.7 in response to refinement so you may wish to delete the sentence at the top of p.7 or clarify it slightly?
- Page 7 of 63, How will you stay informed section: In everyday English, a 'local authority' is, for example, Manchester City Council - you need an alternative term here.
- Page 7 of 63, small typo: participate **in** national meetings
- Page 7 of 63, small typo: How will you refine section: incorporate**d** - or maybe 'provided'

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 11/05/2023 to 06/06/2023

The following amendments were approved by the executive committee.

3.1. Amendments to Project Licences

[redacted], Understanding Vision & Developing Therapies for Blindness.
[redacted], Neural Basis of Tactile Behaviour.
[redacted], Brain Networks for Memory & Executive Function in Health & Disease.

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

[redacted] Generation of Flt4$^{-/C51W}$ 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.

4.2. [redacted] cannot attend the meeting in July so requested that a deputy is sent in his place. The Chair approved this and the committee agreed it was appropriate when given assurances that the applicant would engage at the pre-AWERB stages and attend any meetings with the BSF staff.

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. It was noted that the Home Office has not provided feedback on any of the submitted Standard Condition 18s. The BSF have raised this with them. A meeting will take place of the BSF compliance group to ensure no Project Licence holder is waiting to hear on an outcome of a SC18 to carry on work where this is not required.

5.3. ARMIS is now set up to flag if researchers are getting near to the animal use limits.

5.4. It was noted that a couple of the SC18s had not been submitted within the required 72 hours reporting period. One was due to a misunderstand by a Personal Licence Holder and the second was reported on the day of the incident but fell over a bank holiday leading to a discrepancy in the time it had taken to report the incident.

5.5. A discussion took place on if the death of a pregnant mouse during measurement of tail-cuff BP could have been avoided. It was felt this could not have been expected and that the restraint used is a light touch and does not usually stress out the animals.

5.6. Clarification was requested on what an air heating tube was and why this was used instead of a heated water bed. Going forward the heated water bed will be used to maintain body temperature of the mice.

6. Any other business

6.1. Breeding mice up to 15 months

AWERB were told that the Home Office advise that animals on a breeding protocol can now be kept until 15 months instead of the previously advised 12 months. The BSF reported that they will inform AWERB on any adverse effects if animals are kept for 15 months.

6.2. Contingency planning following cyber incident

AWERB were informed that there are no ethical or welfare concerns at present for the animals following the cyber incident. The BSF are working closely with estates to ensure lights and environmental controls stay in place. Alternative systems are being put in place for ARMIS and ORACLE that require the VPN which has currently been removed by the university.

It has not been discussed as a possibility that a large scale cull of animals would be needed as was seen during the Covid lockdown.

6.3. Podcast

The Podcast is planned for the September 2023 AWERB meeting. [redacted] will take this forward.

6.4. Home Office audit

The Home Office will undertake a Facilities Audit of the BSF on 10th October 2023. It is not a full system audit like the recent one that took place.

The next meeting will be on 20 July 2023 at 10am-12.30pm.

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