Animal Welfare and Ethical Review Body (AWERB)

11.00 26/4/2024, in [redacted] and by Teams

Minutes

Present:	[redacted]	Chair
	[redacted]	PEL Holder
	[redacted]	NACWO, NIO, NTCO, HOLC
	[redacted]	NVS
	[redacted]	Scientific Member
	[redacted] (via Teams)	Scientific Member
	[redacted]	Lay member
	[redacted]	Lay member
	[redacted]	Clerk

Apologies: None.

STANDING ITEMS

- **Apologies for absence**
  None.
- **Quoracy**
  The quorum was met.
- **Declarations of interest**
  None reported.
- **Minutes**
  No prior minutes as this was the first AWERB meeting under the current PEL. Committee agreed to proactive publication of redacted minutes on BRF web page, in line with good practice under the Concordat on Openness on Animal Research.
- **Matters arising**

  Terms of reference

- Reference to EU Directive needs to be removed.
- [redacted] questioned whether 'promote awareness of animal welfare' should be reworded. Committee decided that 'promote' should remain as is because this is how it is worded in the Guidance.
- Membership section should list roles of members.
- TOR to be converted to proper SGUL format.

Action agreed: [redacted] to convert TOR to SGUL format and implement revisions.

Project licences under consideration

- **PPL application for additional availability**
  Received and considered/approved/noted: [redacted]

  Animals under this PPL currently held at [redacted] following closure of BRF at SGUL in 2021.

  Decision made: Committee agreed to approve amendment subject to minor revisions:

  - Remove reference adverse effects and humane endpoints for fin clipping in Protocol 3 as there is no fin clipping in this protocol.
  - After oral clarification from [redacted] on steps in protocol 4, anaesthesia codes are incorrect. AA/AB should be removed and must be changed to AC in all steps.
  - Address minor spelling and grammar issues.

  Action agreed: [redacted] to meet with [redacted] and [redacted] to review changes. Application to be submitted following approval from [redacted] and [redacted].

- **PPL application for additional availability**
  Received and considered/approved/noted: [redacted]

  Animals under this PPL currently held at [redacted] following closure of BRF at SGUL in 2021.

  Decision made: Committee did not approve application for additional availability due to multiple concerns with the application. The primary concerns to be addressed were:

  - Authorisation to transfer fish from protocol 1 to 2 is missing.
  - Protocol 1 refers to 'fin clipping of larva >5dpf (AA)' as in the ASRU template for standard protocols. As the template is incorrect, this needs to be rectified to 'fin clipping of larva <5dpf (AB)'.
  - Step 1 in protocol 1 and 2 could include production of GAA by creation of founders and breeding of GAA. Otherwise, mild GAA would have to be bred in the moderate protocol 2.
  - Video observation needs to consider good practice, e.g., habituation to setup and visual isolation. As this may be repeated, a maximum number must be set.
  - The wording sometimes mentions "small", and other elements evocative of larvae. The opposite would be required to monitor the movements of adult fish. The wording should be reviewed to adapt to all developmental stages, as well as the adverse effects, otherwise a limit of the developmental stages must be set.

- Protocols overall seem to jump between harmful phenotypes and killing before fish are allowed to suffer. This is a fine line and would require considerable care to prevent breach of the terms of the licence.

- Some answers need to be reconsidered, such as to the question: “Will your experimental design be determined by a regulatory guideline?”

- There is a lack of reasoning for classification of protocol 2 as moderate severity. It was questioned whether the protocol has been defined as moderate ‘just in case’.

- There is a lack of specificity regarding adverse effects and how they will be monitored, e.g body condition scoring.

- There is little indication whether fresh tissue samples are required and in which condition. This would help to request authorisation, for example, to decapitate larvae under terminal anaesthesia.

Action agreed: [redacted] to meet with [redacted] to address concerns. Amended PPL application to be circulated to AWERB electronically for comments. Application to be submitted if Committee is satisfied with revisions and approval is granted.

Updates to the AWERB

- **NACWO report ([redacted])**

  - Wildtype zebrafish were imported from [redacted] on March 20th. The NVS conducted an inspection on March 27th.

  - Daily monitoring confirms the fish are growing at an appropriate rate for their age. However, a number of juvenile fish exhibited minor spinal deformities near the caudal fin. Affected fish are and will be euthanized using a Schedule 1 method.

  - [redacted] confirmed similar deformities in fish from the same batch. To establish a healthy founder population, we imported new wildtype zebrafish on 16th April.

  - Continuous rotifer cultures were introduced to the facility with all three cultures showing high density. Rotifers are fed to larvae from day 5 post fertilisation, promoting natural preying behaviour.

  - Both fish racks and the tank washer were serviced on April 5th and are in good working order and a brand-new rack has been purchased and will be installed on 29th May.

- **NTCO report ([redacted])**

  - Four Personal Licences of our fish users [redacted] were successfully transferred to SGUL from UCL in late March.

  - The Personal Licences of all animal technicians have been activated in early April.

  - All Fish Users will need to complete an Induction before they bring fish in the facility. This can be arranged by contacting myself or the Principal Animal Technician.

  - All Training and Competencies will need to be reassessed at a local level before commencing any procedures and Schedule 1.

Action agreed: [redacted] to arrange inductions for fish users before they begin working in the facility.

- **NIO/HOLC report ([redacted])**

  - A new version of the SC18 form (Standard Condition 18) will be released soon. The Home Office are currently developing an updated submission form.

- The standard response time for new Standard Condition 18 notifications is 2 weeks.

- **NVS report** ([redacted])

  Report from site visit attached (Appendix I).

- **3 Rs**

  - [redacted] is appointed as '3 Rs Champion' and will be driving 3 Rs initiatives in the future.

  - It was noted that the current focus is to establish a good founding population, so trials will likely not commence until this has happened.

  - Alternative genotyping methods are a priority;

    [redacted] has a Zebrafish Embryonic Genotyper (ZEG) and is interested in exploring this as an option. [redacted] noted issues reported by other facilities when using the ZEG for lines with multiple alleles.

    [redacted] favoured 3dpf fin clipping over ZEG as DNA sample is larger. [redacted] agreed to arrange training.

    Skin swabbing was discussed as a potential refinement, however [redacted] raised concerns regarding fish welfare due to lack of anaesthesia during emersion and restraint.

  - [redacted] asked about potentially having an internal newsletter from the facility. [redacted] happy with the idea but explained that time constraints have interfered with rolling this out.

Action agreed: [redacted] to explore 3dpf fin clipping and ZEG as genotyping options and will arrange training of BRF staff.

Other business

*Starred items not discussed unless notified to the Chair or Clerk in advance of the meeting.

- ***Dates of Future Meetings**

  Doodle poll to be sent out by Clerk.

- **Any Other Business**

  - Lay members requested a tour of the facility.

    Action agreed: [redacted] to arrange time for facility tour.

  - [redacted] suggested adding a statistician as a point of contact for the AWERB.

    Action agreed: [redacted] to contact statistician to arrange.

Appendix I. NVS report from visit 26/4/2024

Facility development:

- The only present animals were AB fish from [redacted] bleached in SGUL:
  - 5wpf on system 1: Twelve 3.5 L tanks of 15-17 fish with DoF 20MAR24 fed brine shrimp and SAFE Caviar 100-200.
  - 10dpf on system 2: Twelve 3.5 L tanks of 50 larvae with DoF 16APR24 fed rotifer. The tanks receive water drops allowing recirculation.
  - Both systems held the 5wpf larvae until the 10dpf were set on system 2.
  - The 5wpf show spine deformities, which was also detected at [redacted]
  - Systems are monitored daily for ammonia and nitrite.

Water parameters

- The water temperature in system 1 has been re-adjusted to prevent too high temperatures.
- Hardness and alkalinity are currently at about 3-4 drops each. This is acceptable but might be low for hardness. We will assess further once the systems are more occupied and according to users' feed-back regarding intra-ova injections.
- We discussed measuring nitrate daily with the Horiba probe as the scores for nitrogen toxicants should start to increase now.

Feeds

- We discussed ways to feed fish on system 1 more. They currently receive 4 meals a day 2 or 2.5 hours apart. Two options were evoked: feed 5 meals a day, two hours apart. The second option would also solve the problem of the lack of 3.5 L tanks. The fish are wild types and do not need to be split in 3.5 L. Indeed, it is better for all to keep these fish in larger volumes of water. They can therefore be regrouped and set in groups of about 40 in 8 L tanks. In these tanks, they can be fed 4 times the current quantity they receive in each 3.5 L tank.
- The feeding regimen currently evolves constantly as the technicians adapt to the new facility and fish. This is expected and good practice. We need a few generations of fish to establish a routine and potentially introduce new/other diets, unless we diagnose a significant lacking with the current feeds we use.

Schedule 1

- Schedule 1 SOPs are in place.
- The three technicians have been assessed as competent.
- There is a process in place to record mortality.
- The next step is to adapt the documents to ensure users can comply with the local requirements and meet their scientific needs. Two points to consider:
  - Make sure the animals are killed promptly by ensuring the pH of the euthanasic solution is >7.7;
  - The range of authorised completion methods can be increased to allow the harvest of intact organs of interest. For example, the ASPA schedule 1 requires to confirm the permanent cessation of the circulation. This can be achieved by severing major blood vessels, meaning that exsanguination would happen promptly if the heart was to beat and the blood would not be able to circulate. Two options are preferred to achieve this in zebrafish: decapitation and the amputation of the caudal peduncle.

3 rapid actions to take:

1. Measure Nitrate on both systems with the Horiba probe.
2. Feed more as discussed above.
3. SOP on schedule 1: add other practical means to complete the killing by severing major blood vessel and confirm the permanent cessation of the circulation.

Actions for future/potential developments:

- Diet trial.
- Standardising the quantity of distributed feeds.
- Adjusting hardness.