

**3Rs subgroup for the
ANIMAL WELFARE AND ETHICAL REVIEW BODY**

Minutes of the meeting held on 8 December 2021

Present:

[REDACTED]

Apologies:

[REDACTED]

In attendance:

[REDACTED]

1. Minutes

Agreed: That the minutes of the meeting held on 29 September 2021 were approved.

[REDACTED] was not listed as attending the last two meeting however was in attendance.

2. Responses regarding queries raised from previous reviews

2.1. [REDACTED], mid-term review of Drug discovery for parasitic helminths [REDACTED]

Discussed:

- The use of a positive control was queried by [REDACTED] and [REDACTED] was asked about it via email. [REDACTED] said that when they test new compounds they aren't using a positive control group, and will only include a positive control for drugs that are working. The group were pleased with the response from [REDACTED] and how the positive control is being used.

3. Retrospective reviews of Project Licences requiring licence holder attendance at meeting

3.1. [REDACTED], Modelling therapies for renal malformations.

Considered: A completed Retrospective review form and presentation

Interviewed: [REDACTED]

- Discussed:*
- The group discussed with the licence holder if the animals used in the work were from both sexes. The two genetic diseases being studied affect both sexes equally clinically therefore the animal work does use both female and male mice.
 - The group asked if the frog work was to reduce work in mice. It was explained that this work was done to learn more about the physiology of the disease without using mice but gene therapy work would not be possible in the frogs.
 - The licence holder explained that funding was only obtained for the mice work even though the original grant application had contained mice and frogs, however alternative sources of funding, for example from the NC3Rs, would be explored in the future as they may be more open to fund this type of work.
 - The licence holder discussed how the tadpole physiology of the urinary tract differs from that of the mouse and human, being more of a tube rather than having a bladder for storage of urine. One way in that they are similar is the presence of glomeruli in the kidney of frogs, mice and humans.
 - The potential disruption to neonates removed from their mothers for injection were discussed. The licence holder reported no rejection of the pups when placed back with the mothers. The neonates are covered in bedding to mask the smell of the handling and placed back in the cage at a distance from the other neonates. The mother will put the neonate back with the litter mates. The group felt it was important that this information was disseminated to other researchers doing injections in pups (to put researchers in touch).
 - The use of homozygous mice for breeding was raised. The licence holder said that they have never tried to breed the homozygous mice.
 - The licence holder had reported the use of neonates or juvenile mice as a refinement. The group discussed that this would not be a refinement from the NC3Rs perspective; refinements are about animal welfare.

3.2. [REDACTED], Healing Mechanisms of Thermal Injury Wounds

Considered: A completed Retrospective review form and presentation

Interviewed: [REDACTED]

- Discussed:*
- It was explained to the group that the work was a multi-centre study where the University of Manchester was the fourth element of five. Due to delays in the previous centres less work was carried out in Manchester than planned.
 - The group discussed with the research technician involved with the work if pigs are easy to work with and if they are singly housed. The research technician explained that they are group housed when they first come into the unit and singly housed the night before surgery and then afterwards. The pigs can see each other through the bars of the cages. As they grow they are provided with a living area and a sleeping area and have toys in the cages too.
 - The research technician explained that it was relatively easy to redress wounds in the pigs when needed, either while the pig was

conscious or by using a mild anaesthetic when more than a small shift in the dressing had occurred.

- The group asked for confirmation that the work was being prepared for publication which was given. The work is currently under embargo but will be published.
- It was noted that the number of animals requested for use on the licence was higher than those used. It was explained that when applying for the licence a number of other experiments were planned however these did not get funding therefore the work did not go ahead.
- It was explained to the group that the dressings were kept in place through the application of a number of layers of materials and then the pigs were covered by a surgical stocking.
- The group are looking at an ex vivo model.

4. Mid-term reviews of Project Licences not requiring licence holder attendance at meeting

4.1. [REDACTED], Designing therapeutic and diagnostic nanotechnologies for medicine

Considered: A completed mid-term review form

- Discussed:*
- It was noted that the licence is large and a number of different models are being used.
 - The group asked the NTCO if the number of Standard Condition 18s related to the size of the licence and the type of models the licence allowed. A number of the Standard Condition 18s were unexpected.
 - An amendment was made to the licence regarding the study which combined small graphene oxide with a small peptide, ovalbumin leading to hind-limb paralysis. Other licences have made an amendment following the unexpected adverse effects.
 - Death of animals under anaesthesia was discussed by the group, and that while these are unforeseen any pattern of deaths would be flagged up on any licence and investigations made as to the cause.
 - The group felt that breeding under the licence was well controlled and there was no evidence of over-breeding.
 - The group noted that no Appendix 1 was included with the report as mentioned in question 6. This will be asked for from the licence holder.
 - The group discussed that the scientists making up the substances are fully aware that they are being injected into animals.

Outcome: The subgroup supported continued work on this licence.

4.2. [REDACTED], Zebrafish models of haemorrhagic stroke.

Considered: A completed mid-term review form

- Discussed:*
- The group thought it was the most clearly completed form they have seen which will be fed back to the licence holder. The licence holder will be asked by the Chair if they are willing for their form to be used as an example.

Outcome: The subgroup supported continued work on this licence.

4.3. [REDACTED], Understanding peritoneal repair and internal scarring

Considered: A completed mid-term review form

- Discussed:*
- The group discussed that the research team are very experienced in terms of the surgical procedures being performed which are not straight forward. Members of the research team are either clinicians or vets.
 - The group is also engaged with members of the BSF.
 - The work being carried out on the licence is closer to the clinic than a lot of other studies have been.
 - The group felt the Standard Condition 18s had been appropriately dealt with.
 - It was felt that some of the refinements listed should have been happening as standard and were therefore not really refinement *per se*.
 - The group discussed the death under anaesthesia and the amendments.

Outcome: The subgroup supported continued work on this licence.

5. New NC3Rs Skills & Knowledge Transfer Award – [REDACTED] - Developing an in vitro model of metastasis

- [REDACTED] gave a presentation on the work she is doing on her NC3Rs Skills & Knowledge Transfer Award.
- The award is jointly funded by the NC3Rs and CRUK. The aim of the award is to transfer a piece of equipment which is currently being used to study the gut by collaborators [REDACTED] [REDACTED] is working to validate this equipment in order to study metastasis in breast cancer, which would replace the use of some animals.
- The group were extremely interested to hear about this new model, and commented that they thought other groups in Manchester would be extremely interested in hearing about it. [REDACTED] and [REDACTED] will help to facilitate presentations, internally and externally, to both cancer research and other groups to help highlight the potential of this equipment. [REDACTED] will work with [REDACTED] to develop a new 3Rs case study for the UoM website.

6. Use of carbon dioxide for Schedule 1 – [REDACTED]

- The group were given a presentation by the NVS explaining that both mice and rats are still being killed with CO₂ at the University of Manchester whereas other establishments have moved away from this method.
- While it is a Schedule 1 method there are some known issues with it.
- CO₂ is very aversive to breathe and animals do struggle to breathe when it is given. At high levels it can become an acid which is painful. At lower levels studies have shown that anxiety is induced by CO₂.
- Alternatives were discussed including anaesthetising with inhalation anaesthesia before exposing the animal to CO₂, i.e. the animal is asleep when CO₂ is given, or using an overdose of injectable anaesthetic such as pentobarbitol.

- The NACWOs present explained their experiences of using CO₂ and if the groups that are using it can be identified and asked to use alternatives where possible.
- [REDACTED] will raise the issue at the next BSF management team meeting, and will look into putting together information on alternatives for both researchers and technicians.
- It was agreed that this topic would be discussed at the next AWERB away day to make AWERB members aware of the issues and the preference to move away from using it.