Royal Veterinary College
University of London

**Summary Minutes:** AWERB minutes

**Status:** FINAL

**Meeting held:** 8 November 2023 at 10am via MS Teams

**Present:** 9 plus 1 in attendance, 2 by invitation and 8 apologies

**1 NEW PPL AT RVC – SEEKING SECONDARY AVAILABILITY**

The PPL Holder was welcomed to the meeting. It was explained that she was working closely with a research group at the RVC on a grant that was investigating pain mechanism in Paget's disease. The project uses transgenic mice that have a Pagetic phenotype and would evaluate pain behaviours with a pharmacological study at the RVC. Her group specialised in pain research and the RVC group in bone diseases so it was a good collaboration to study pathological bone pain, as pain was often the most debilitating side effect of skeletal diseases. The PPL Holder was therefore seeking secondary availability at the RVC. This licence would expire in March 2024 so a new replacement licence was also being written.

There would be several protocols carried out at the RVC:

- Breeding for mild or moderate phenotype.
- Acute pain behaviour (to test what the sensory and pain thresholds are for mice).
- Inflammation (to test the effects of anti-inflammatory/analgesic agents on recurring pain measures, e.g. a pharmacological study where pain behaviour can be measured at baseline and after drug administration
- In vivo electrophysiology

The licence was discussed and the following queries were raised:

- **Swim test**: the licence referred to swim tests with the temperature of the water being kept at 23-26°C. RVC's recommendation was alternatives to this test should be considered. Its use had been discussed at a recent AWERB Hub Workshop and the consensus had been that the test should be replaced as it was not a good model of depression and was a cause of significant stress for animals. Furthermore, the temperature of the water seemed too cold and cold temperatures could induce depression in an animal. There was also a risk that an animal might stop moving to try and maintain its body temperature and not use so much energy. The PPL Holder explained that this temperature had been chosen following advice from her NVS that the temperature should be the same as the room temperature. However, although the test was mentioned in the licence, they had not used it for several years as their research was currently focused on different aspects.
- **Noxious mechano-sensation: the licence mentioned that this could be carried out in 3 different ways. Was it planned to use all 3 options?** It was confirmed that it was as they were trying to understand how the animals recapitulate the human phenotype as that would strengthen the translational value of the work.
- **Four different routes of administration were mentioned in the licence including intramuscular, intradermal and intraplantar. It was not clear though which volume corresponded to which route?** This question was raised particularly in relation to the intraplantar route of

administration. The PPL Holder advised that when this licence had originally been written the Home Office Inspector had recommended a generic volume to provide flexibility. For the new licence though, the PPL Holder would be more specific on volumes and routes of administration, as it was already known which drugs would be injected and by what route, as they had recently received a program grant to carry out specific studies.

For the intraplantar injection it was usually 20 microliters into one paw. A baseline test would be done and then depending on how the thresholds changed after the injection, another test done two hours later to check the thresholds. The animals would be monitored over a few days if it was a long lasting drug like an antibody.

- **For two of the protocols, one of the humane end points related to body weight loss. It was not clear though how often the mice were monitored for this?** Weight loss was monitored at least weekly. Sometimes it was done more often, especially after a surgical procedure. The focus was on not disturbing the animals too much though and to keep them in their home environment where they were comfortable. A lot of time was spent with the mice, to acclimatise them so they got used to being gently handled by the same people. The aim was that when the pharmacological study started, the mice were comfortable, and there could be confidence that the study was not being influenced by external factors.
- **One of the protocols included a table with many different inflammatory agents. Was the plan to use any of these at the RVC? Some of the substances listed had limitations in terms of which routes can be used but there was no specification about that**? The PPL Holder confirmed that they would not be aiming to induce inflammation in the Paget mice, as that was already an established pain state in the mice.
- **The pain phenotype in these mice was not characterized, so presumably that was the first step that needed to be done? How obvious was the phenotype and how easy would it be to do the behavioural tests and to look at analgesic drugs?** It was recognised that a phenotype would be needed to see if any of the drugs have an effect.
- **For the testing it was mentioned that the mice would be subject to multiple acute sensory tests in order to reduce and refine the numbers of animals used for assessment and that there would typically be three testing days per week. Would the tests be conducted on consecutive days or would they get at least one day off after each test day?** The frequency of the tests was dependent on whether it was a pharmacological study and, if so when the peak drug effect occurred, as they needed to ensure that they did not miss any critical time points.
- **How was the frequency of the general anaesthesia (GA) for the imaging of two times a day, five times per week and 10 times per month determined? Two times a day seemed to be excessive.** This had been based on the experience of other PIs.
- **A query was raised about one of the protocols and why it was needed, if the plan was not to use agents to induce inflammation.** They had transgenic mice that were insensitive to pain so they were aiming to understand whether the genes that conferred the transgenic phenotype were involved in regulating inflammatory pain or were regulating other kinds of musculoskeletal pain. Changes were needed to this section though in the new licence.
- **Was the secondary availability just being requested for the Pagetic Mice or for other mice too?** It was just for the Pagetic Mice as that is what they had funding for. The academics wanted to strengthen their collaboration in musculoskeletal pain and would potentially be jointly applying for future grants. It was helpful to have experts in different aspects coming together to study the different symptoms of the disease pathogenesis and that by working together they could generate some really impactful data.

The PPL Holder was thanked for attending the meeting.

Once the PPL Holder had left, AWERB were asked for their views on the project licence. The consensus was that the PPL Holder was very experienced in this area and had thought a lot about the project.

As a new project licence was in the process of being written to replace the current licence that was due to expire in March, and as there were no major problematic issues that immediately needed to be addressed, it was agreed that no changes were required to the current licence. Instead, feedback would be put together focussing on changes that needed to be incorporated into the new licence, and aspects of the licence that needed to be made clearer, so that these could be taken into account during the new PPL review process. AWERB's concerns about the animals being anaesthetised twice a day would be included as well as a request that alternatives to the swim test be looked into.

One area that did need to be covered was that if work under the licence progressed to the point of assessing different analgesic strategies, then it was important that the assessor of the responses to the evoked and behavioural tests was blinded to the treatment that the mice received. The requirement for this would be stressed and that this would need to be included within the study approval form.

Another area of concern was the health status of the Pagetic mice. The researchers needed to make sure that they could obtain them in the future. They were an important model so needed to make sure the strain was not lost.

**2 PPL AMENDMENT**

An amendment to a project licence had been submitted. The PPL Holder was not at the meeting as both the NVS and NACWO were supportive of the proposed amendment and were able to talk through why it was required. The requested change was to modify the subcutaneous administration option within one of the protocols to enable changes to be made to the routes of administration. This would enable some flexibility in terms of dosing. This change would be beneficial both to the welfare of the animals and also the science.

A query was raised about why this option had not originally been included in the project licence. It was explained that an oral therapeutic drug had been developed but had turned out to be quite unpalatable and difficult to administer. Work was being done to improve the flavour and change the concentration but it had become clear that to ensure that the full drug dose was being delivered that it needed to be through a subcutaneous administration.

On hearing that both the NVS and NACWO were supportive of these changes, AWERB confirmed that they were content for the project licence amendment to be submitted to the Home Office.

**3 MINUTES OF PREVIOUS MEETINGS**

The minutes of the meeting held on 18 October 2023 were confirmed as an accurate record.

**4 ANY URGENT ITEMS TO RAISE?**

**4.1 Update on sheep study**

AWERB were reminded that at the previous meeting they had been given an update on a sheep study involving laryngeal implants. One of the sheep had deteriorated following a recovery surgery and had been sent for a post-mortem. A decision had now been made to pause the work until after Christmas so that time could be taken to sort issues out. A meeting had been arranged for mid-November, which one of the NVS would chair, so that the researchers could present their data and analysis and discuss the next steps.

**5 DATE OF NEXT MEETING**

This was scheduled for 6 December 2023.

Secretary
14 November 2023