UNIVERSITY OF CAMBRIDGE
University Biomedical Services

Compliance Assurance Meeting

To [redacted]

[redacted]

From [redacted], Named Information and Compliance Support Officer

Date Friday January 26th

Time 13.00

Venue [redacted]

Subject [redacted] Mice Ear Pinna A & B

Pages 6

Our Ref [redacted]

Policy-

Compliance Assurance Meetings (CAMs) are a management tool, designed to ensure Governance outside of the Act, to protect individuals, ensure due process and systems are implemented within the division of University Biomedical Services. This process will ensure the University upholds the highest standards of animal welfare and good practice relating to animal care and use.

NOTE: A CAM meeting does not replace the investigation of an incident by the Home Office or other authority. Therefore for example, where the local inspector deems appropriate a Home Office non-compliance investigation may run in parallel or sequentially with a CAM.

Event- 3 events [A) and B) only] incidents occurred within a short time space under PPL [redacted]

A) On a routine veterinary inspection of the [redacted] facility, [redacted], NVS observed that mice had large areas of their ears missing, (PPL expected adverse effects specify that one hole 1-2mm may occur in the SAME pinna as the inoculation), some breaking through the pinna edge to leave flaps and some almost completely destroying the pinna (see Appendix 1-3). This investigation of the cause and post procedural observations is being undertaken to understand:

i. why this adverse outcome has occurred,
ii. what the mice experienced to get like this,
iii. why it was not reported by users or staff,
iv. how/why the science lead to this with

the aim being to prevent it happening again.

The experiment (study plan [redacted], attached) performed by [redacted] under protocol 3, involved 2 cages of C57/B6 female mice, 6-8 weeks old, 5 mice per cage. These mice were purchased externally from the usual commercial supplier. The mice were injected intradermally in the pinnae of the ear with 10^4 plaque forming units of either wild type vaccinia virus strain Western Reserve (WR) or a mutant virus vDeltaF14 (in which the F14 gene of the parent virus had been deleted).

Personal Licensees visit the facility to perform procedures one to two times per day at approximately 0900 and 1700, to avoid use of the single changing station whilst technicians require the use of it. Members of the research group perform all mouse procedure health checking and leave a note for the UBS facility staff if they note any changes in or concern about an animal. The research licensees are self-trained and are instructed by UBS staff not to re-use needles, which UBS supply free of charge.

[redacted] found the 5 mice infected with wild type virus, 4 had lesions that exceeded the adverse effects lesion size described in the project licence. Of the vdeltaF14-infected mice 2 out of 5 exceeded this size. In total this means 6/10 mice developed lesions in their ears that exceeded those expected following inoculation by this route and with this type of virus.

The virus titre administered was checked by back titrating the infectious dose actually injected into the ear pinnae.

[redacted] was asked by [redacted] to conduct a dose ranging experiment with the same wild type virus used in the mice described above. The titre of virus administered would be reduced in 2 fold steps from the standard **10^4** titre and the mice carefully monitored to identify whether scientifically valid data could be obtained at a lower titre that did not induce any adverse effects.

These animals were subsequently euthanased without retention of tissue or post mortem.

B) Mice in a study (24/08/2017) conducted by Personal Licence holder [redacted], using protocol 1, was discovered by [redacted] (Animal Technician) who reported animals with holes in their ears, the edges of which appeared to be scabbing. She reported this to [redacted] (17/01/2018) after her morning check because the mice appeared to have developed the same type and size of lesion, post inoculation, as those described in A above. [redacted] inspected and assessed each mouse during the senior afternoon check and confirmed [redacted] observations, noted the mice where not showing any other clinical signs before she reported the existence of these mice to [redacted] who inspected the mice (19/01/2018). This study used study plan [redacted] [attached]. At this stage [redacted] (PPL) requested that intradermal injections cease until further investigations have been completed.

The Home Office Inspector [redacted] has been informed of all developments by [redacted] Deputy Director.

Attachments:

Project Licence documentation

[redacted]

Study Plan documentation

[redacted]

Outcomes-Events A and B only

This Compliance meeting was held to determine why parts of animal's ears were found missing by the NVS (Event A) and an animal technician (Event B), how this happened, and where the responsibility for observation of animals lies.

UBS staff undertake the welfare and daily husbandry care checks for the animals and observe effects which, in the case of this group, have nothing to do with the scientific, adverse effects of the study. The latter are monitored by the researchers. [redacted] researchers undertake daily or twice daily checks on their animals; normally at times when the one hood in the containment area is not required by UBS staff. This was discussed and [redacted] indicated that she had spoken with her staff and agreed that they should more thoroughly check all mice and not leave science related health monitoring entirely with the researchers.
Any adverse effects either health or research related should be notified and flagged by both scientists and UBS staff, some clarification and guidance will be provided by UBS. **Action 1**

[redacted] was aware of his responsibility but said his focus had been on observing and measuring the primary inflammatory lesion which forms following intradermal (i/d) injection (primary lesion expected by day 5, reaches peak size by 10 to 14 days after which it begins to resolve and is fully healed by 3 weeks). Therefore once the primary lesion had resolved he had stopped his measurements and detailed observations. He had noticed that when the scabs dried and detached, holes developed in the ears where the scabs used to be. In his view apart from missing tissue the ear had healed. He therefore considered there had been no adverse effect as the primary lesion had not exceeded the 6mm size allowed in protocol 3. The primary lesion size is measured using electronic digital callipers under general anaesthetic.
Some confusion was highlighted regarding under which protocol the procedures related to, [redacted] clarified:

1. Event A – This experiment, now completed, was undertaken on protocol 3 – This was an acute experiment where holes in ears were not expected because animals are killed before scabs are lost and holes appear.
2. Event B – This experiment, still in progress, was undertaken on Protocol 1: Following primary infection by the intradermal route mice are kept for 28 days when they are challenged intra-nasally (i/n). Holes in ears are expected in experiments where animals are kept for challenge. However there is a mismatch between the size of the holes seen in some experimental animals and the description of expected hole size in the adverse effects section of protocol 1.

[redacted] to oversee and amend PPL [redacted] adverse effects sections of protocols 1 and 3 require amendment to more accurately reflect the expected adverse effects that can occur (primary inflammatory reaction vs the scab/necrotic area which develop may result in a hole when the scab detaches) and to provide clear end points. **Action 2**

Clarity was sought regarding the size of the acute lesion, the area of the scab over the lesion and deficit once the scab had dropped off from the ear pinna. [redacted] confirmed that the size of the scab plus, when present, the adjacent area of necrosis and the subsequent hole should be the same. [redacted] informed the meeting that he did not measure the hole once the scab had dropped off. He said any signs of pain, suffering and distress were not observed, immune response was

complete and activity normal. [redacted] said that during the acute phase local scratching may be observed but is not recorded. [redacted] said just because these animals did not show systemic signs did not mean that they did not feel pain. Localised pain would be unlikely to precipitate systemic signs of piloerection and hunching which are described in the adverse effects section. [redacted] indicated that whilst she was taking photos of the mice with ear lesions the mice seemed reluctant to prick their ears forward. Subtle monitoring of local reactions and recording of ear position could be used to develop score sheets which might be useful as a monitoring tool for UBS and research staff. [redacted] reminded the researchers that the adverse effects section contained end points which are procedure related and these end points need to be adhered to even when the overall severity category assigned to the protocol is higher. In other words just because the severity category for protocol 1 is severe, animals should not be allowed to reach this level of suffering if this is not expected for a particular step or procedure. Prey animals will only show subtle clinical signs unless they have advanced disease and for this reason observers will need a keen eye. [redacted] noted the recommendation for additional monitoring of animal well-being particularly during the period when the acute lesion is present, such as measuring degree of scratching and any other evidence of local irritation and pain.
The group will discuss health monitoring with [redacted] the optimal way to do this and will review observation and measuring frequency. **Action 3**

[redacted] noted that animals were now being more closely monitored at all times by UBS staff and asked how many animals remained on these experiments. [redacted] tated that 15 animals are currently infected under B) with 15 mock infected animals as controls. [redacted] said an additional 10 animals have arrived for a dose ranging study which has not yet commenced. This latter experiment will start at a dose 2-fold lower than the normal dose used and reduced down to 100-fold lower than the standard dose. [redacted] suggested [redacted] should seek advice from the HOI with regard to lesion size and endpoints. Strains and mutants from the dose ranging study should provide data to inform the amendments made to protocols. [redacted] showed a graph which illustrated how, using a standard procedure, the maximum lesion size has changed in the last 10 years. He said this was probably due to the way the lesions are measured.
A form of monitoring which reduces operator variation would be advantageous. [redacted] suggested the group might investigate an alternative digital soft wear program used by health care professionals for measuring cutaneous lesions which might prove more accurate than using callipers. **Action 4**

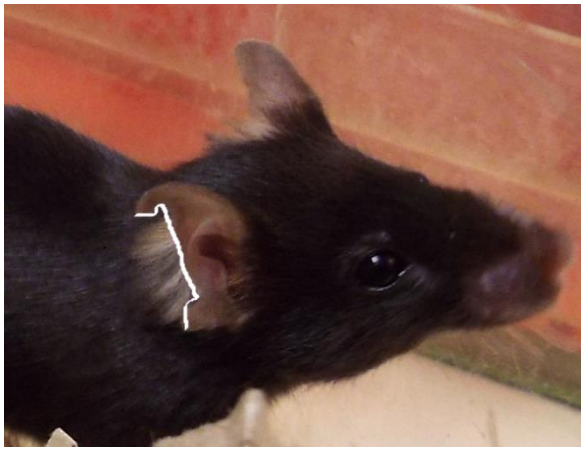
[redacted] informed the meeting that Study Plans (SPs) had been a subject for discussion, there is a failure of Technicians to engage with them due to the technical and scientific nature of the content, which was considered too complex. It was felt these should be written in a way that all staff can understand them and work with them for the benefit of animal research and animal welfare.
Study plans should be written in plain English, additional guidance will be provided centrally from UBS. **Action 5**.

[redacted] advised the meeting that SPs should be drawn up for each experimental. The contents of the SP should be checked against the licence authorities (Project Plan: Part D and Protocol: Part E) to ensure authorities exist. During the experiment if something occurs the SP and the licence should be consulted and appropriate action taken. At the end of each experiment the SP could be used as a template for the next experiment but the authorities and adverse effects should be reviewed and the SP updated, if necessary. In some cases it may be necessary to complete project

licence standard condition 18 reports and in some cases a project licence amendment may be required before work continues.
Study plans should make clear which steps of a protocol are to be used (e.g. write the protocol step number next to the relevant step in the study plan) to ensure the SP agrees with the licence authorities. SPs should be reviewed after an experiment ends and if another similar experiment is required update the SP as necessary. **Action 6**

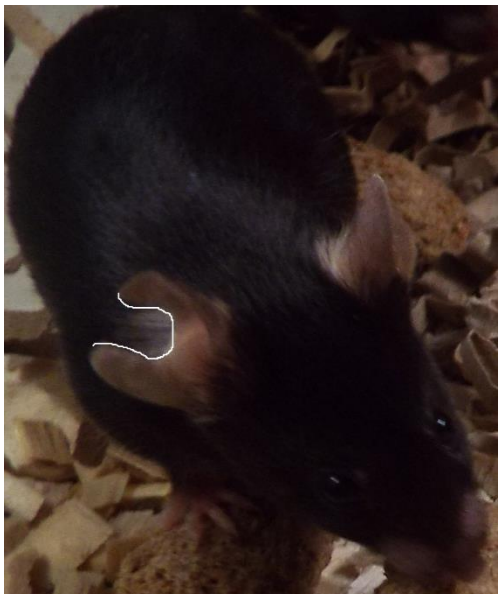
Actions
Action 1: Any adverse effects either health or research related should be notified and flagged by both scientists and UBS staff, some clarification and guidance will be provided by UBS.
Action 2: [redacted] to oversee and amend PPL [redacted] adverse effects sections of protocols 1 and 3 require amendment to more accurately reflect the expected adverse effects that can occur (primary inflammatory reaction vs the scab/necrotic area which develop may result in a hole when the scab detaches) and to provide clear end points.
Action 3: The group will discuss health monitoring with [redacted] the optimal way to do this and will review observation and measuring frequency.
Action 4: A form of monitoring which reduces operator variation would be advantageous. [redacted] suggested the group might investigate an alternative digital soft wear program used by health care professionals for measuring cutaneous lesions which might prove more accurate than using callipers.
Action 5: Study plans should be written in plain English, additional guidance will be provided centrally from UBS.
Action 6: required before work continues.
Study plans should make clear which steps of a protocol are to be used (e.g. write the protocol step number next to the relevant step in the study plan) to ensure the SP agrees with the licence authorities. SPs should be review after an experiment ends and if another similar experiment is required update the SP as necessary.

Appendix 1



Appendix 2

Appendix 3



Glossary of terms

AA	A procedure performed without anaesthesia
AB	A procedure performed under anaesthesia, from which the animal recovers
AWERB	Animal Welfare and Ethical Review Body
CAM	Compliance Assurance Meeting
HOI	Home Office Inspector
NVS	Named Veterinary Surgeon
NACWO	Named Animal Care and Welfare Officer
PIL	Personal Licence (Procedures Individual Licence)
PILh	Personal Licence (Procedures Individual Licence) holder
PPL	Project Licence (Procedures Project Licence)
SC	Standard Conditions (of either PIL or PPL)
UBS	University Biomedical Services