



PPL Standard Condition 18 Notification Form

Please use this form to notify the Animals in Science Regulation Unit (ASRU) of procedural-related adverse effects that have exceeded or are likely to exceed the severity limitations or controls described in the project licence. ASRU will use this information to determine whether any further action is required.

Do not use to report:

- adverse effects that are due to non-procedural issues;
- potential non-compliance (report by other means);
- issues without adverse welfare consequences.

If you are in doubt whether or not you need to fill in this form, please contact your assigned Inspector.

Please clearly title your document: '**ASRU_establishment name_PPL number_PPLh surname_date_sc18**' and send to your Inspector (e.g. using cjsm) with the subject heading 'PPL SC 18 Notification PPL [Number]

Establishment Licence name	University of Cambridge
Establishment Licence number	X81BD37B1
Name of PPL holder	Maria P. Alcolea
PPL number	PP7037913
Protocol no	06
Protocol title	Changes in epithelial cell behaviour over time (Moderate) (Mice)
Severity classification	Moderate

1. Date of incident	Sunday 27 th March 2022
2. Species	Mouse (M00765708)
3. Brief details of study and how the limits or constraints have been breached	In study plan SP126688, two litters (13pups) are administered a transgene inducing agent (tamoxifen) by subcutaneous injection at postnatal day 6, and 13. The aim of the study is to harvest these animals at different time points through adulthood to investigate the effect of Sox9 in the development and maintenance of homeostasis in the oesophagus squamous stratified epithelium. One of the pups started looking slightly hunched on 26th April and had the lowest weight of the litter, for that reason it was under close observation and it was decided that if the condition did not improve within 24h it would be culled. Unfortunately, this pup was found dead the following morning (on the 27th April, at day 18 after birth and 5 days after the last subcutaneous injection of tamoxifen). The body and tissues of this animal could not be analysed because when it was found it had already been cannibalised.
4. Have you taken advice from the Named Animal Care and Welfare Officer/Named Veterinary Surgeon/others?	Yes, we had a previous meeting with the NVS to discuss about the adverse effects encountered in this line (K14Cre-So9flox) with Tamoxifen. Together we designed a strategy to reduce the volume and dose of tamoxifen in future experiments, and amended the licence to include subcutaneous route administration in all protocols as a refinement. For this study we followed those refinements and had reduced the number of injections and spaced them in time to allow pre-weaned animals to recover.

5. Cause of Problem, if known	Animals receiving certain genetic-inducing or deleting agents (such as tamoxifen) may experience weight loss 1-2 days after administration. The likely incidence of this occurring is dependent on the genetic strain of the mice, but is not uncommon. These mice typically regain weight back to baseline within the next 2-7 days and display no harmful side effects. Although we were not able to analyse the corpse as it was cannibalised, the last recorded weight of this pup was lower than the rest, suggesting that it had not been feeding well. It is likely that the weight loss after tamoxifen injection and insufficient feeding caused the death of this animal.
6. Action that has been or will be taken	The rest of the pups in this study have low weight compared to untreated animals, so they have been supplied with diet gel and are being closely monitored, their body weights recorded typically every other day. Given the difficulty to evaluate weight changes in pre-weaned animals, due to the rapid variation in this parameter, animal welfare at this stage is being assessed daily by using a body condition scoring method. Animals harvested at short time points after tamoxifen induction will be used to assess the impact of tamoxifen injection and gene deletion in the general function and morphology of the tissues. These observations will be shared to the NVS to discuss if any adjustments are needed for that protocol and particular line.
7. Reported by (name)	Maria Teresa Bejar Serrano, animal found by Mia Watkins
8. Date	29/03/2022

*****For official use*****

Inspector's comments and recommendations:

Comments and recommendations	
Inspector name	
Date	