Road Map Resource Pack

FOCUS ON SEVERE SUFFERING

PART 3: ONGOING REVIEW

1st edition. March 2016

RSPCA Road Map resource sheet 3 – to complete as part of the retrospective review

Actual severity of protocols

Project licence number	
Date granted	
Date of review	

Protocol #	Protocol title	Predicted severity	Number of animals used	Actual severity*

*If actual severity data has not been formally assigned at the point the retrospective review is done, this column can be used to indicate the estimated severity as a percentage for each of the categories

RSPCA Road Map resource sheet 3 – to complete as part of the retrospective review

Actual severity of protocols

Project licence number	7076/54
Date granted	May 2015
Date of review	June 2017

Protocol #	Protocol title	Predicted severity	Number of animals used	Actual severity*	
1	Pharmacokinetics	Mild	180	Mild Moderate Severe	180 0 0
2	Behavioural assessment	Mild	520	Mild Moderate Severe	518 0 2
3	Telemetry and cardiovascular assessment	Moderate	80	Mild Moderate Severe	20 60 0
4	Experimental stroke	Severe	50	Mild Moderate Severe	0 40 10

*If actual severity data has not been formally assigned at the point the retrospective review is done, this column can be used to indicate the estimated severity as a percentage for each of the categories

RSPCA Road Map resource sheet 4 – to complete as part of the retrospective review

Focus on refinement

Project licence number	
Protocol number	

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might make it worse?**	**What actually happened?**	
	Adverse effects and indicators of these	**Were the predicted adverse effects observed?**	**Did the refinements applied help to reduce or avoid suffering?**

Note: This example is intended to give an indication of some of the points and factors that could be discussed when conducting this part of the review. It is for guidance only and is not intended to be exhaustive for this type of procedure.

RSPCA Road Map resource sheet 4 – to complete as part of the retrospective review

Focus on refinement

Project licence number	7076/54
Protocol number	4
Experimental stroke	

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might make it worse?**	**What actually happened?**	
	Adverse effects and indicators of these	**Were the predicted adverse effects observed?**	**Did the refinements applied help to reduce or avoid suffering?**
Pre-operative training on behavioural tests over a 2-3 week period: bilateral sticky label test (for contralateral neglect), beam walking (for hindlimb coordination) and staircase test (for skilled forelimb paw-reaching)	Minimal stress/ anxiety can be caused before animals have habituated to the tests, as testing involves moving animals to novel rooms/arenas	Initially yes but this improved with improved habituation/training protocol.	Yes, once the pre-test habituation protocol was modified to reduce the stress associated with the behavioural test equipment/room.
Food restriction (85-90% of free feeding weight) pre-operatively and from 7 days post-MCAO to facilitate performance on staircase test	Mild hunger; possible frustration and anxiety	No clinical signs of anxiety or frustration were noted and no animals lost more than 10% of their initial body weight.	N/A - In this case, the potential welfare concern was not seen and no further action was needed.
Under general anaesthesia, transient (90 min) occlusion of the MCA using an intraluminal thread advanced via the common carotid artery	Pain and discomfort associated with surgery Potential for unexpected surgical complications, e.g. subarachnoid haemorrhage, ipsilateral retinal injury, intraluminal thrombus	In initial studies, some issues were seen and some animals (6/10) were humanely killed in the post surgical phase due to endpoints being reached. Improvements to surgical technique and intensive post-operative care	Although this protocol is classified as severe, we believed that it should be possible to run it with no more than moderate severity. Once the refinement practices were fully optimised we were able to reduce the actual severity to moderate for

	formation, brain oedema hypothalamus involvement with consequent hyperthermia or temporal muscle necrosis. These can present in a number of different ways, for example – sudden collapse, paralysis, severe head tilts, seizures Aversiveness and potential effects of anaesthesia on physiological variables (such as hypothermia, hypotension, hypoxia) Poor nutritional intake resulting from reduced consciousness level, impaired mastication and poor motility, generally in the first 48h post MCAO Degree of locomotor deficit, which could cause stress and/or frustration	meant that these issues were overcome and subsequent studies were much more successful with the endpoint being reached in 4/40 subsequent surgeries.	most animals going forward.
Behavioural tests (bilateral sticky label test and beam walking test) undertaken daily from day 1 to day 28 post-MCAO; staircase test undertaken daily from day 7 postMCAO	Animals may find the tasks stressful if their motor abilities are compromised	Yes, initial studies, some animals showed signs of stress. This was lessened by reducing the test duration.	Yes, the change to test duration reduced the impact of distress.
Administration of novel therapeutic agent by s.c/ i.v/ i.p. route before and/or after surgery (prophylactic/therapeutic)	Transient discomfort associated with administration route No adverse effect expected at the dose levels administered	No issues were observed	Yes, institutional guidelines on administration of substances were followed and all animals were closely observed following dosing.

Longitudinal MRI under anaesthesia on days 1, 7, 14 and 28 post-MCAO	Repeated anaesthesia Aversiveness and potential effects of anaesthesia on physiological variables (such as hypothermia, hypotension, hypoxia)	Some signs of aversion were identified in a proportion (20%) of animals that were exposed to repeated imaging. This was most likely due to the learnt aversion to the anaesthetic used (isoflurane) and further analysis is being conducted to address this issue.	No. Awaiting results of further analysis of studies to date to try and understand why some, but not all, animals experience aversion.

Note: This example is intended to give an indication of some of the points and factors that could be discussed when conducting this part of the review. It is for guidance only and is not intended to be exhaustive for this type of procedure.

Slide 1 Road Map resource pack: Part 3; Retrospective review.

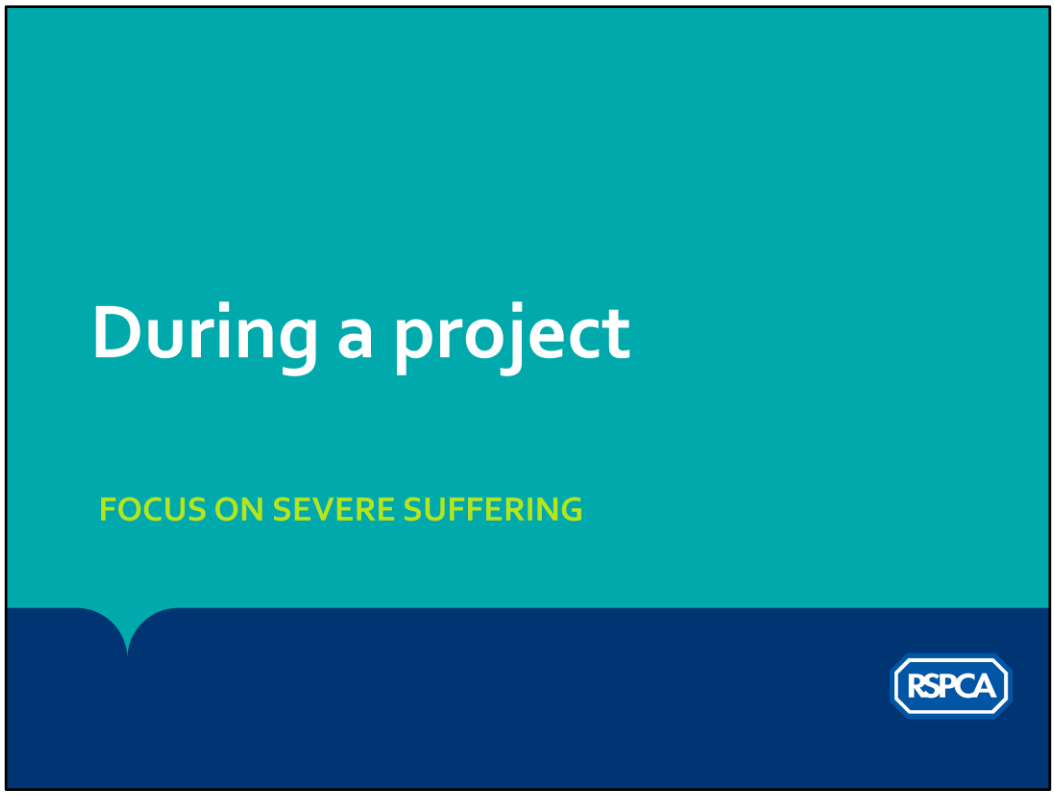
This set of slides was prepared by the Research Animals Department of the RSPCA, and is intended primarily as a practical guide for Animal Welfare and Ethical Review Bodies (AWERBs) or other institutional animal care and use committees, to establish a mechanism towards reducing and avoiding severe suffering within their establishments.

The resource is intended to be accessible to all members, each of whom may have participated on the AWERB or committee for some time, or may be relatively new to their role. Some members may thus be very familiar with the information and approaches set out in these slides, whereas the materials, technical details and processes mentioned will be less well known to others.

Each slide has associated notes which provide a guide to the points you could make while giving the presentation, but the intention is for you to use your own words rather than read the notes as they are.

Please read the Guidance for Facilitators before giving this presentation.

You can contact the Research Animals Department if you would like to receive an editable version of this resource or any additional information: research.animals@rspca.org.uk



Slide 3.1 Reviewing procedures that have the potential to cause severe suffering

This series of slides aims to guide the AWERB (or other similar body) through a retrospective review of *ongoing* projects and offers an opportunity to consider how much severe suffering has occurred and how effective the current refinement approaches are.

N.B. These slides follow-on from **Part 1** of this resource which introduces the **'road map' approach and principles** and **Part 2** which covers **prospective review** of severe procedures.

In this resource, we will use the term '**retrospective review**' to describe the process of the review of *ongoing* projects.
Resource **pack 4** will deal with' **retrospective assessment**' at the *end* of the project.

Retrospective means: 'looking back on or dealing with past events or situations' and can occur during or at the end of an event or series of events.

Retrospective review is defined on page 89 of the Home Office Guidance to ASPA and is the practical implementation of one of the key tasks of the AWERB to 'follow the development and outcome (retrospective review) of projects'.
Chapter 6 (page 32) of the RSPCA/LASA Guiding principles on good practice for animal welfare and ethical review bodies outlines some principles of retrospective review and is a useful resource to refer to.

Slide 3.2 Why retrospective review of ongoing projects is important

The revised ASPA requires the AWERB to:

'follow the development and outcome (retrospective review) of projects carried out in the establishment, taking into account the effect on the animals used; and to identify and advise on elements that could further contribute to the 3Rs'

In practice this means that the AWERB should follow the progress of all projects with the aim of improving both animal welfare and the quality of science. Some form of formal process needs to be in place to ensure this and retrospective reviews may be implemented at one or more points in the life of a project or at regular intervals if there are specific issues that need monitoring. Severe suffering may be one issue that warrants more frequent review.

In addition, the legislation also requires that precautions must be made to 'prevent or reduce to the minimum consistent with the purposes of the procedure any pain, suffering, distress or discomfort that may be caused to the animal' and that personal licencees are required to 'act at all times in a manner that is consistent with the principles of replacement, reduction and refinement'.

Therefore, there is an imperative to find ways to reduce suffering (apply refinement) wherever possible and this extends beyond the initial project application.

Slide 3.3 Reviewing welfare assessment records to see whether severe suffering has occurred

Directive 2010/63/EU requires the assessment, and reporting of, actual severity experienced by individual animals. This means that there should be a process in place, in every establishment that performs regulated procedures, to use welfare assessment records to determine how much suffering every individual animal experiences.

If actual severity assessments are not available at the time of the review, the cage-side welfare assessment records can be used instead.

This process is an opportunity to review the frequency, duration and origin of severe suffering in a project and to identify opportunities to reduce or avoid it going forward.

N.B. Although there is no formal requirement to make the actual severity assessment as procedures are progressing, it is likely to be easier to do this rather than at the point when the data is requested by the Home Office.

More information and guidance on how to perform actual severity assessment can be found on the following slide and here:
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf
And here:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/276014/NotesActualSeverityReporting.pdf

Slide 3.4 Checking the materials required for a review of an ongoing project

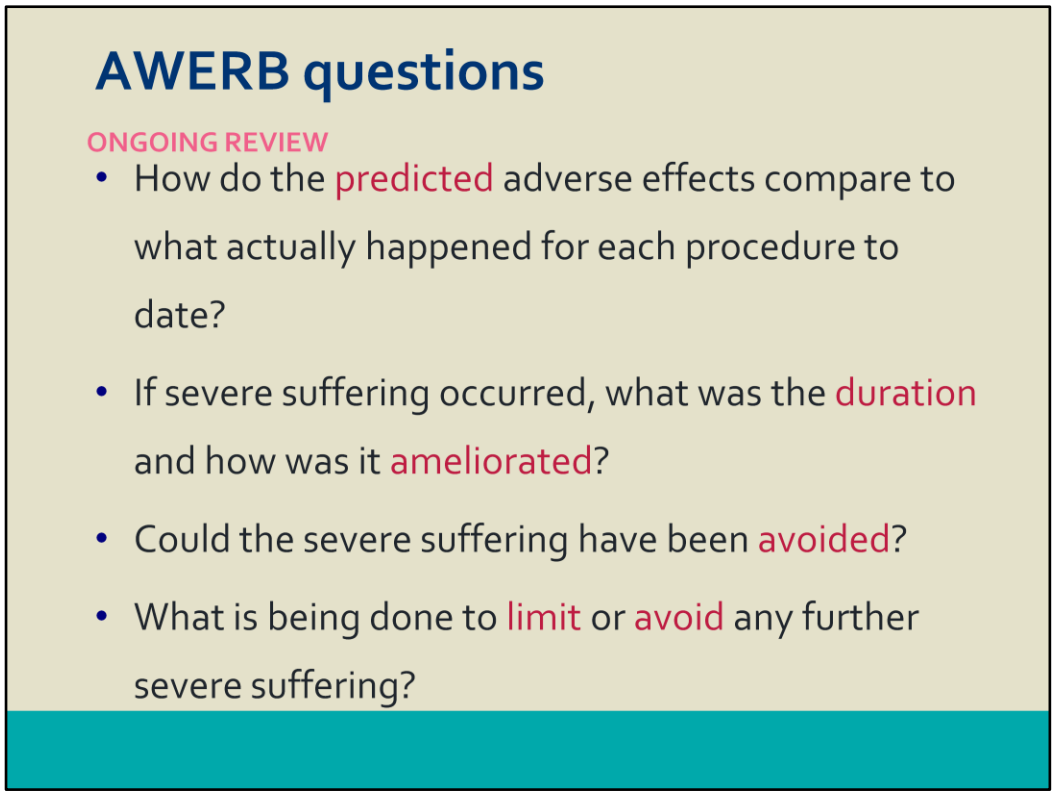
We are now going to review the project in question, to see whether severe suffering has occurred, understand the reasons for this and to review approaches to avoid or reduce it.

We will use

•The project licence application form
•Records from the prospective review of the project (see part 2 of this resource) if applicable
•Summary information about the experimental outcomes thus far in the project which should also include the cage-side welfare assessment records and the animal 'fate' (i.e. what happened to animals at the end of the procedure).
•There may be additional information that may be useful to the review; for example, any staffing issues (a key animal care giver may have been off sick during a set of experiments and humane endpoints may not have been applied as thoroughly), equipment failure (a fault with temperature control may have resulted in physiological stress), infection in the animal unit (this may cause an unexpected increase in actual severity in procedures that are usually no more than moderate) or any other factor that may affect the severity of procedures.

It is important that the review takes into account input from all staff that have been involved in the project so far. This should include the Principle Investigator/Lead researcher, project licence holder, NACWO and NVS and any key scientific or technical staff.

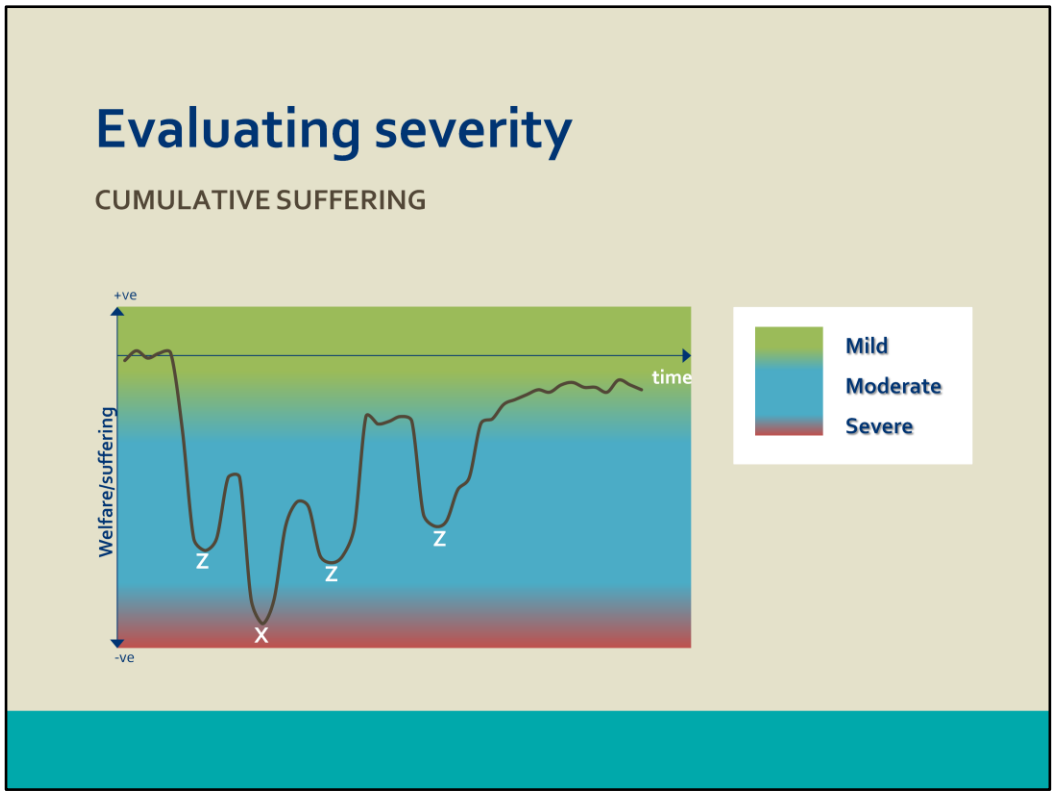
[Before beginning the review, it would be helpful if members of the group conducting the review read:
•Section 6 of the RSPCA/LASA Guiding Principles, which addresses retrospective assessment and retrospective review, and
•Chapter 5 of the RSPCA Lay Members' Handbook, which addresses reviewing project applications.]



Slide 3.5 AWERB questions

With all of the relevant information brought together several questions need to be asked. In essence, the ongoing review needs to assess whether and when severe suffering has occurred thus far in the project. This may have been predicted (the procedure may have been prospectively assessed as severe) or it may have occurred unexpectedly. In any case, the nature and duration of the severe suffering should be clearly defined and the efforts that have been made to reduce the suffering should also be made clear. It is important to ask if severe suffering could have been avoided in all cases. Could anything be changed to prevent or limit future suffering? Were all of the specified refinements (including humane endpoints) actioned? Was the welfare assessment plan appropriate?

These questions form the basis of the review and will be expanded upon in the following slides.

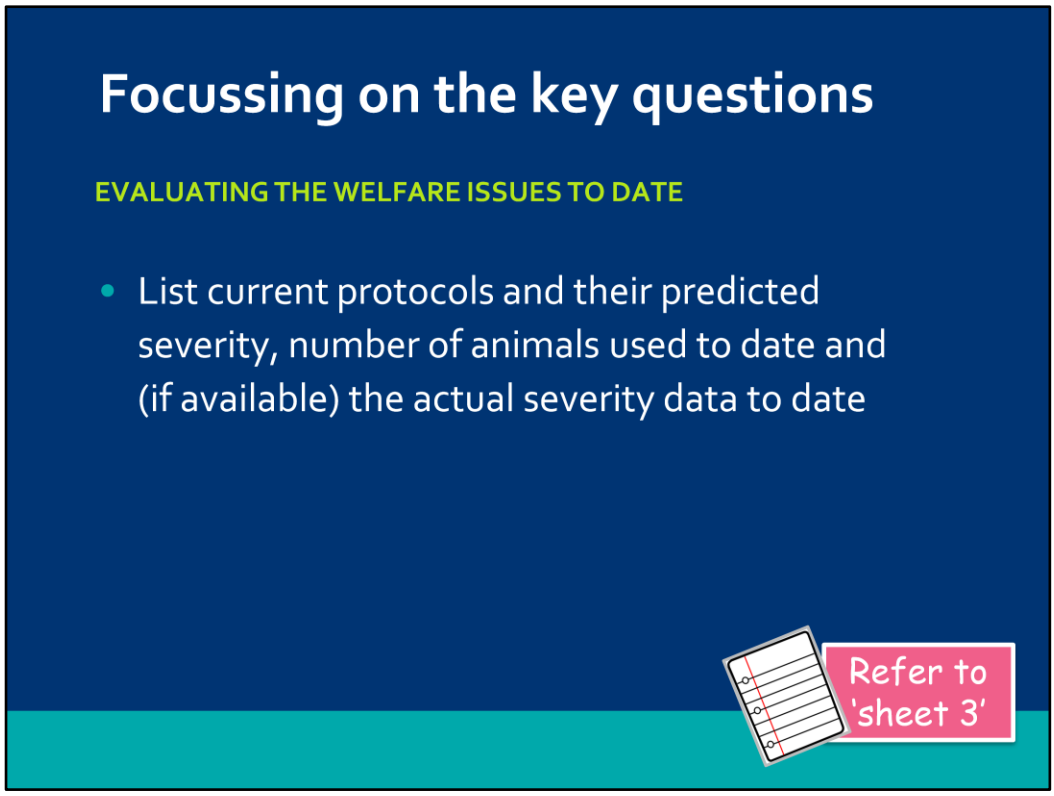


Slide 3.6 Thinking about severity
Although actual severity data is useful in flagging up procedures and time points of concern, it is important to recognise that the assessment of actual severity for reporting purposes involves the worst experience of the animal, not the average or predominant severity. For this review process, it is essential to consider the whole lifetime experience of the animal.

In the graph there is a hypothetical timeline of suffering for an individual animal. The peak of suffering occurs at point x and is in the red 'severe' band. In accordance with the Home Office guidance for reporting of actual severity this animal would be returned as severe.

If we look at the three other highlighted 'Z' troughs we can see that, even though they indicate 'moderate' suffering the duration of these troughs is quite long. Even if peak X didn't cross into the 'severe' band, the overall 'area under the curve' would indicate prolonged moderate suffering and this may (as stated in the Home Office Guidance to ASPA) be regarded as severe suffering. It is important to consider what is happening to the animal, and why, at these stages.

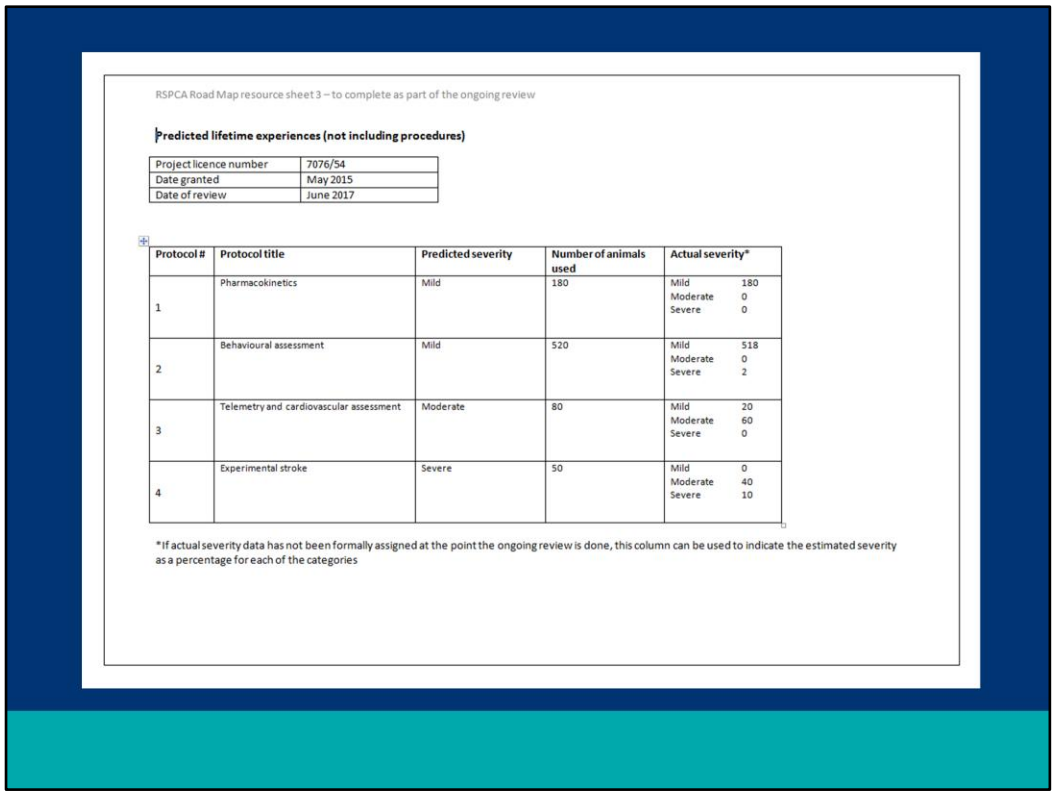
This example illustrates the importance of regular welfare assessment and the potential for cumulative severity.
The take-home message is that severe suffering can occur due to prolonged moderate suffering and that all suffering should be taken into account when determining the actual severity experienced by an animal.



Slide 3.7 Evaluating the severity to date I
For each protocol in the project, it is important to evaluate the nature and degree of suffering that has occurred to date. If formal 'actual severity' data is available this is useful but it should be noted that, as mentioned in the previous slide, this indicates the low-point of welfare for the individual animal and not the whole lifetime experience.

The 'actual severity' is a clear indicator that severe suffering has occurred but does not give any indication as to *why* the severity was this high. The cage-side records and the welfare assessment protocol are much more important tools for the full retrospective review.

Sheet 3 can be used to highlight the 'exposure' to severe suffering to date. As mentioned above this is not the 'whole story' but will quickly show where the focus of the review needs to be. If formal actual severity data is not available or up to date, the cage-side records need to be used to assess the degree of suffering experienced for each animal used in each procedure and specifically to highlight whether there has been any severe suffering.



RSPCA Road Map resource sheet 3 – to complete as part of the ongoing review

Predicted lifetime experiences (not including procedures)

Project licence number	7076/54
Date granted	May 2015
Date of review	June 2017

Protocol #	Protocol title	Predicted severity	Number of animals used	Actual severity*	
1	Pharmacokinetics	Mild	180	Mild Moderate Severe	180 0 0
2	Behavioural assessment	Mild	520	Mild Moderate Severe	518 0 2
3	Telemetry and cardiovascular assessment	Moderate	80	Mild Moderate Severe	20 60 0
4	Experimental stroke	Severe	50	Mild Moderate Severe	0 40 10

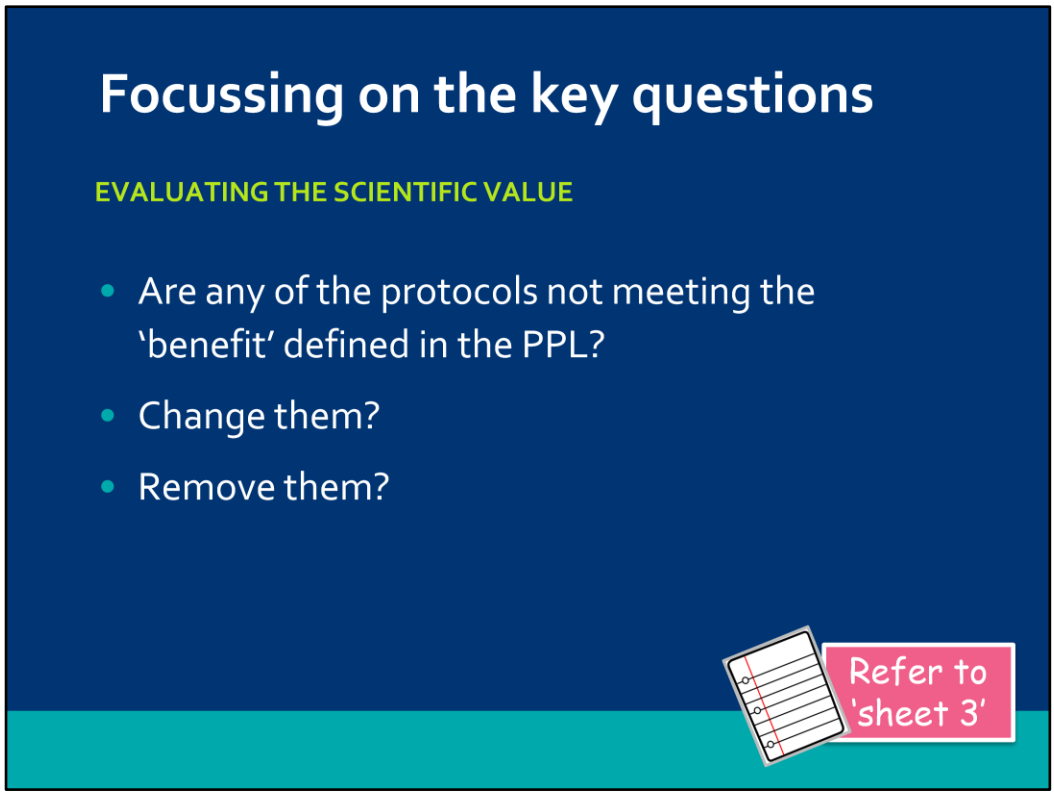
*If actual severity data has not been formally assigned at the point the ongoing review is done, this column can be used to indicate the estimated severity as a percentage for each of the categories

Slide 3.8 Evaluating the severity to date II
This is sheet 3a which has some example data included.

In this example the project licence has four protocols with prospective severity categories ranging from mild to severe. The actual severity data was available and up to date. Overall in the project, 12 animals experienced severe suffering and in the next few slides we will delve more deeply into the causes of this severe suffering and what actions need to be taken forward.

There are two important issues to note with these data:
In protocols 3 and 4, the actual severity was less than the prospective severity which is a good indication as to the effectiveness of the current welfare assessment and refinement regimes. However, some animals clearly did not benefit from the refinement and this offers the opportunity to 'drill down' into the data to try to work out why this was the case.

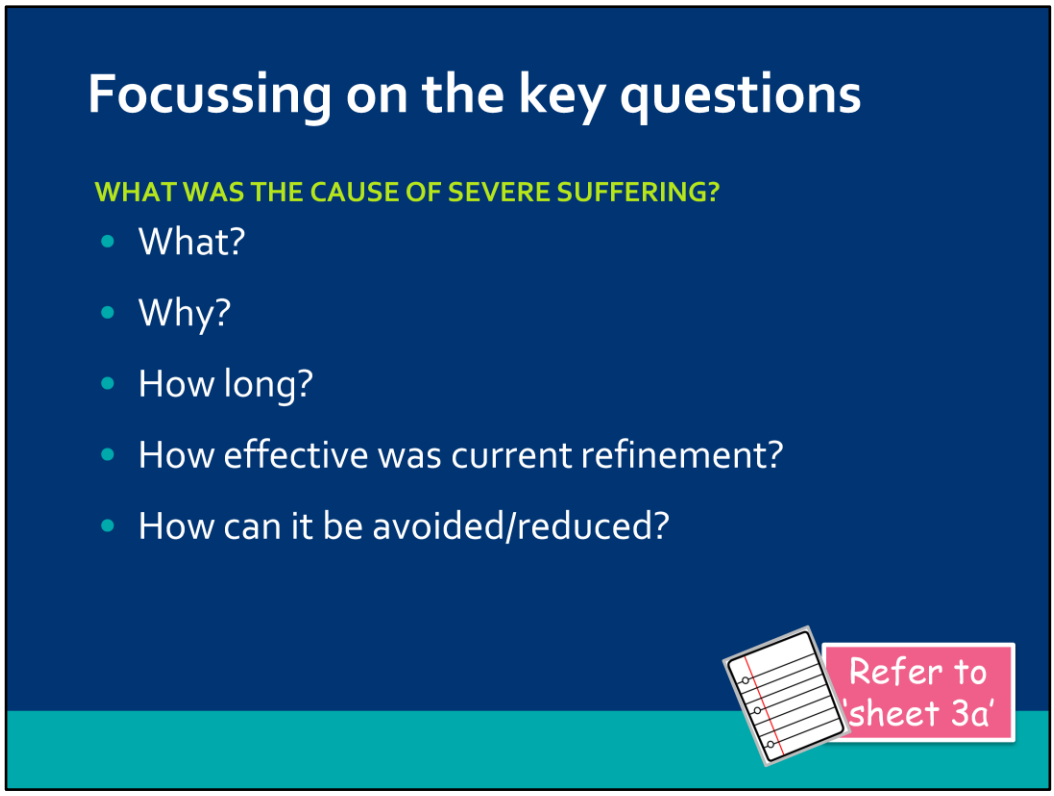
In protocol 2, two animals experienced severe suffering under a mild category protocol. This should have been immediately reported to the Home Office and the cause(s) of this severe suffering identified. We will explore the reasons for this (in this example data set) in the next few slides.



Slide 3.9 Evaluating the science
At the time of the retrospective review, some data should have been generated (arguably it is of much greater value to perform the review at a point when enough studies have been done to provide enough information to review) and this can be used to evaluate how the progress to date maps against the project aims in the project licence.

It may well be evident that one or more protocols are not contributing meaningfully to the overall project aims and this is an opportunity to look more closely at these. If any of these protocols have the potential for severe suffering, taking this opportunity to remove them from the licence because they are not contributing to fulfilling the aims of the project should be considered.

Removing a protocol is not the only way to address the issue; amending the protocol may be possible and this should be considered (this would require further AWERB and HO review and approval). If a 'severe' category protocol is amended this offers an opportunity for further reflection on the justification for the potential for severe suffering and for additional refinement to be considered.

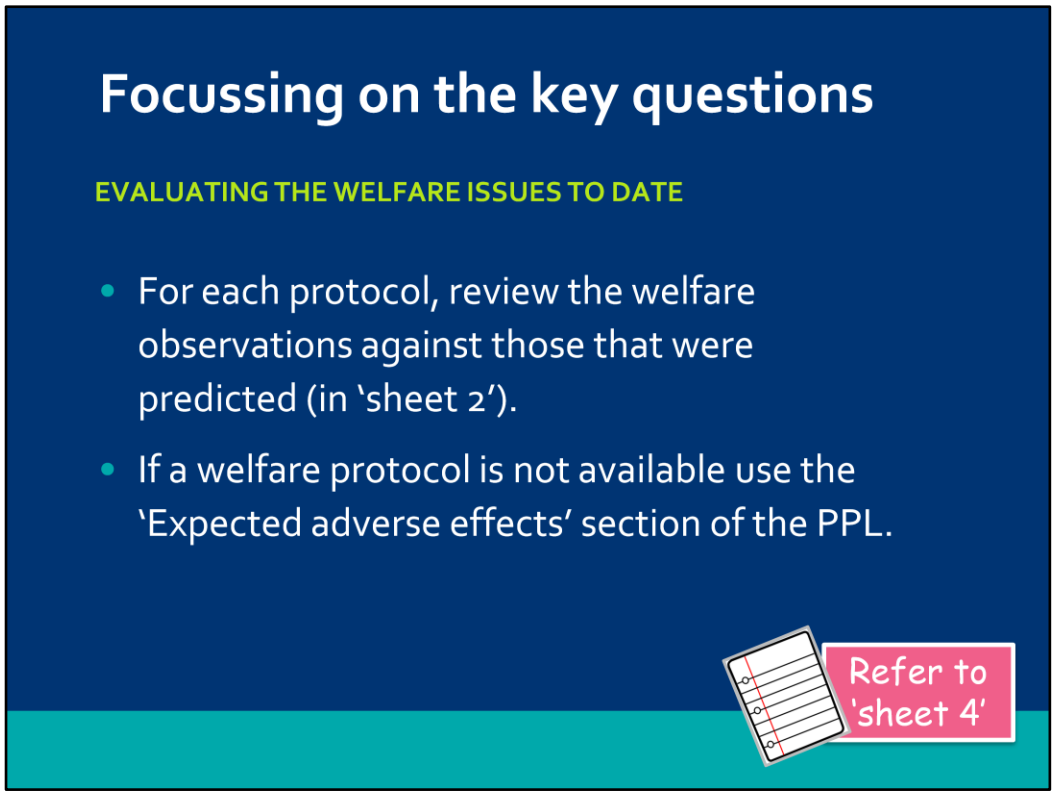


Slide 3.10 Evaluating the severity to date
In sheet 3a, two protocols had incidences of severe suffering.

Protocol 2 is a 'mild' category protocol, but two animals experienced severe suffering. The review process needs to establish the cause of this severe suffering and identify ways to avoid or reduce it going forward. This may require a review of refinement practice and an update to the welfare assessment protocol and cage-side recording.

In this example, the two animals reported as having experienced severe suffering were two mice found dead in their home cages during a morning check. These animals had not shown any clinical signs of ill health, pain or distress up to this point and a post mortem did not indicate the cause of death. Since these animals may have suffered in the hours prior to death their 'actual severity' was classed as severe but this was reported to the Home Office and no further action was needed.

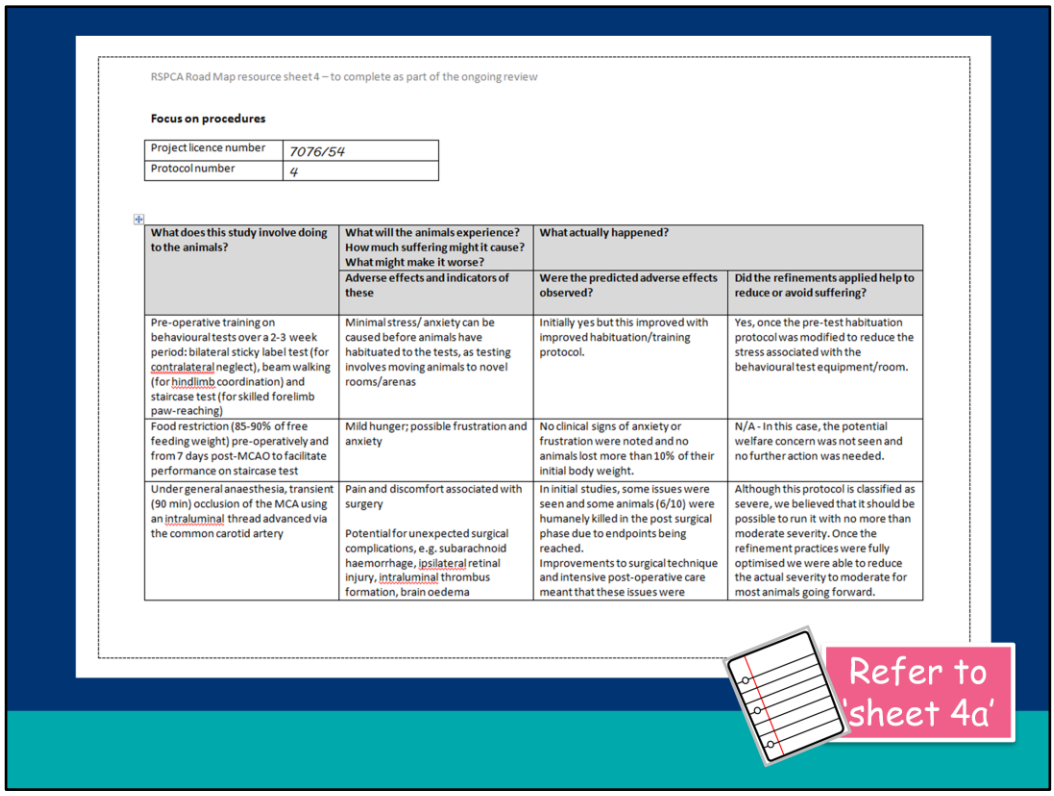
Protocol 4 is a severe category protocol and this necessitates a greater degree of analysis.
Additional analysis should be done with any procedure where severe suffering has occurred or where the welfare concerns that were highlighted prospectively to contribute to severe suffering were observed.



Slide 3.11 Evaluating the severity to date
Ideally, each experimental protocol should have a parallel welfare assessment protocol which, for each step of the experimental protocol, details the potential welfare issues and how to identify and ameliorate them.
Examples of these can be found with part 2 of this resource (sheet 1a and sheet 2a).

As indicated in the previous slide, if a procedure has led to animals experiencing severe suffering or if potentially severe welfare issues were observed, additional analysis is needed. For this we will use sheet 4.
Sheet 4 mirrors sheet 2 for the first two columns ('What does the study involve doing to the animals?' and 'What will the animals experience?') and then has two new columns to address whether the expected adverse effects were observed and whether the current refinement practice was effective or not.

If sheet 2 was not used, the 'Expected adverse effects' section of the project licence can be used to populate the first two columns of sheet 4.



Slide 3.12 Evaluating the severity to date

Sheet 4a is based on 'Illustrative examples of the severity process Model 4 – Stroke' from the EC guidance document on severity assessment (p48): http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf

(The first two columns of sheet 4a have been copied from the EC documents and the outcome information is fictitious).

Expanding on the example in slide 9, here we examine protocol 4 and the reasons for the severe suffering that was identified.

There were several potential sources of suffering in the protocol and one in particular (middle cerebral artery occlusion) that was the source of the severe suffering (10 animals out of a total of 50 used in the protocol to date).

For each of the potential sources of suffering identified in sheet 2, column 3 needs to be populated with information regarding the actual incidence of suffering that was observed. Column 4 allows reflection of the impact of the refinements and endpoints identified in sheet 2.

In this example, the research group aspired to 'run' the model at moderate, despite the severe category (remember this is the worst-case scenario for potential suffering) but their initial surgical technique required modification. Once the initial 'teething' issues were dealt with the incidence of severe suffering reduced (from 60% in the initial phase, to 10% going forward).

This is a significant achievement but it may be possible to reduce this further by further analysis of the welfare records and additional refinement.

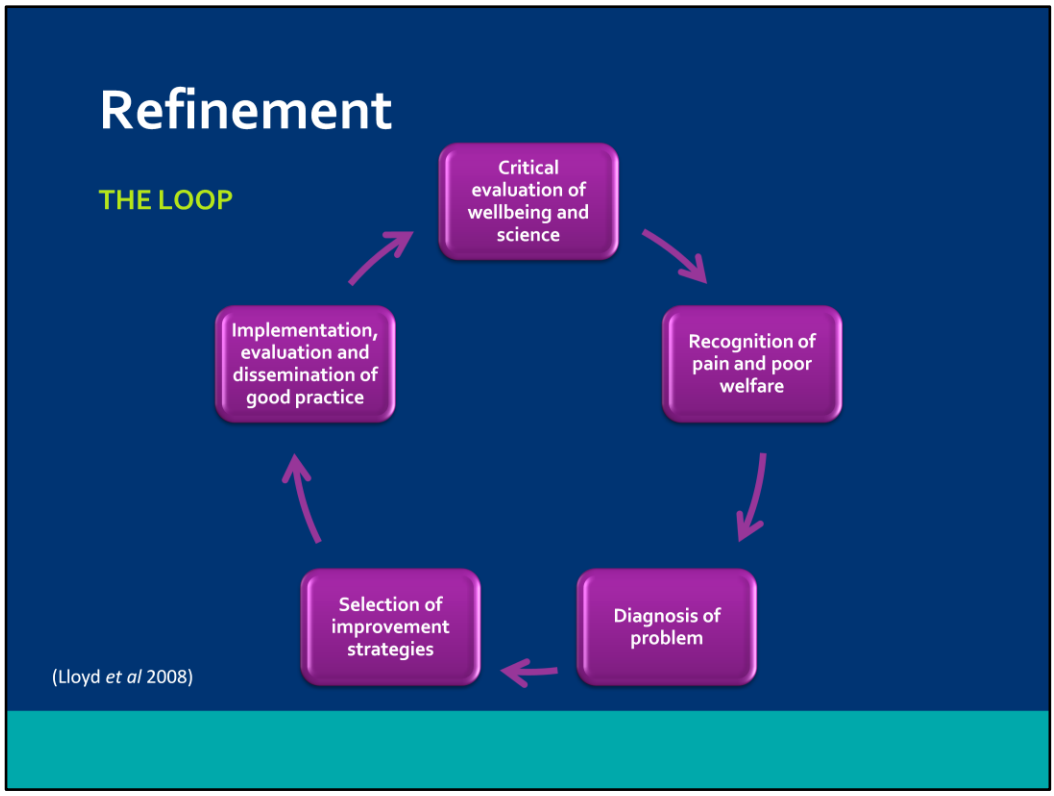
Slide 3.13 Looking to make further improvements
In an ideal world, refinement efforts would benefit all animals in a study to the same consistent extent; this may not be the case, especially when higher levels of severity are possible.

In order to establish why a particular protocol causes higher levels of suffering to a proportion of animals the full life-time experience of the animals needs to be examined and any 'flags' that may indicate the source(s) of suffering identified.

Building on the previous example, the first cohort of animals used in protocol 4 experienced severe suffering because the surgical approach and the post surgical care were not yet fully optimised. In the subsequent cohorts of animals used in this protocol the situation improved massively (the incidence of severe suffering was reduced from 60% to 10%) but was not yet perfect. The purpose of this part of the review is to try and establish what more can be done to make the situation even better.

In this fictional example, further changes to the surgical technique, additional analgesia and changes to the post surgical care could all produce further refinement. Other factors needs to be considered including the pre-study health status, weight, age and stress levels of the animals may have an impact on the welfare impact of the surgery.

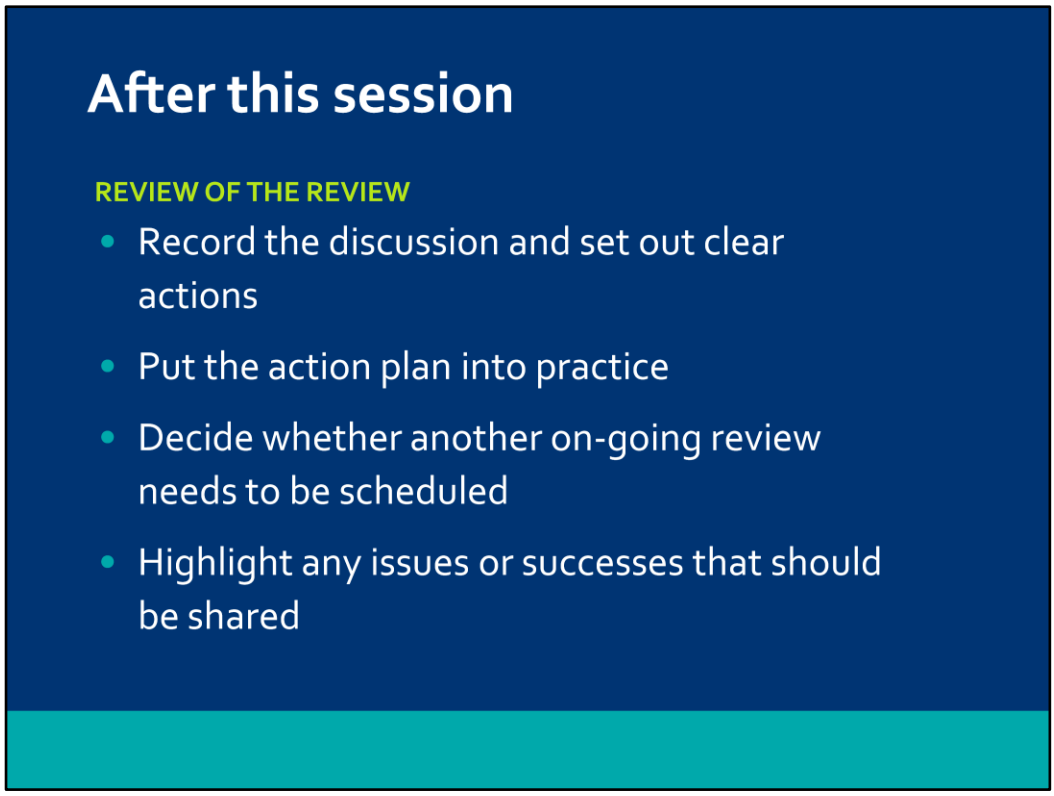
Once potential additions to the welfare protocol have been identified, these need to be implemented and their impact evaluated and monitored.



Slide 3.14 Refinement as an ongoing process
Refinement is an ongoing process that needs to be evaluated and adapted to meet the needs of the animals and to improve the quality of the science.

The application of refinement is a process of welfare assessment, implementation of change, monitoring of the impact and further welfare assessment – in a loop.

It should never been seen as a one-time event during the planning of the study (although it is important that it is an integral part of the planning process).



Slide 3.15 Review of the session
During this review it should have been clear what the extent and causes of suffering have been for a particular project.

It should be clear where refinement practice has been successful and where issues need to be addressed.

There should be a clear action plan to make the improvements necessary to further reduce or eliminate severe suffering and this needs to be carried out.

The AWERB needs to decide how much further oversight they need for the project and if they would like an addition on-going review at a later date.

Finally, any clear findings, positive or negative need to be shared to help raise standards throughout the establishment and, if possible, outside the establishment.

End of the session