Report of the 2013 RSPCA/UFAW Rodent Welfare Group meeting

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Introduction

The RSPCA/UFAW Rodent Welfare Group holds a one-day meeting every autumn so that its members can discuss current welfare research, exchange views on rodent welfare issues and share experiences of the implementation of the 3Rs of replacement, reduction and refinement with respect to rodent use. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents, ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

To mark the 20th anniversary of the Rodent Meetings, the 2013 meeting began with a presentation on animal welfare issues in China, including the work of the RSPCA with respect to laboratory animals (as the 20th wedding anniversary is the 'China' anniversary). Other speakers gave presentations on aversion to euthanasia agents, assessing welfare and reducing suffering in rodent disease models and the importance of understanding rodent behaviour when designing experiments and husbandry – and when interpreting data. All the presentations end with some action points. The meeting also included a special session on welfare assessment in genetically altered (GA) rodents, with talks on current practice and an interactive discussion session.

Animal welfare in China

Paul Littlefair, International Department, RSPCA

Animal welfare is a rapidly evolving field and nowhere has this been more the case than in China over the past decade. There are a number of identifiable factors which have raised the profile of animal protection issues in China in recent years. Internal factors include a catalogue of widely publicised animal abuse incidents, a sharp rise in companion animal keeping, an increase in meat and dairy consumption and animal health crises as well as food safety and security issues. These have all added to the momentum for improvements in the treatment of animals.

The debate has also been driven by external influences, particularly as China's political and economic standing in the world has been boosted and its participation in

multilateral fora has had growing implications for animal welfare. The space for this debate has been shaped by the global social media boom, which has played a major role in exposing China's animal issues to both the domestic population and the international community.

Of all areas of animal use in China, the field that has shown the most potential for integrating animal welfare improvements has been laboratory animal science. The sector has grown rapidly since the impact of economic reforms began to be felt in the early 1980s and the field of laboratory animal sciences is also globalised to an extent generally not seen in other areas of animal use. Since 1999, China has dramatically increased its share of scientific papers published worldwide. Chinese scientists increasingly acknowledge that poor laboratory animal welfare may undermine the validity of scientific results and therefore the quality of the research.

China breeds around 19m laboratory animals a year, compared to an estimated 20m in the US and 12m in the European Union (EU). If the country is to continue to excel in science and particularly in the bioscience fields, then it needs to secure and maintain both scientific quality and competitiveness. These economic and technological imperatives have enabled the RSPCA and others to promote good laboratory animal welfare as conducive to good science.

As early as 1988, the Ministry of Science and Technology (MOST) issued a Statute on the Administration of Laboratory Animal Use which covered the basic needs of laboratory animals and the requirement for trained personnel. This was followed in 1997 by the first explicit appearance of 'animal welfare' and the 3Rs in MOST's Laboratory Animal Development Programme for the Period of the Ninth Five-Year Plan. From 2004, first Beijing and then several provincial laboratory animal administrations also included modest animal welfare references in their regulations. While not laws in the strict sense, these ministerial Statutes and municipal or provincial regulations operate as *de facto* legislation and as such represent the first appearance of 'animal welfare' in China's regulatory measures. This environment has provided opportunities for the RSPCA and others with an interest in laboratory animal welfare to partner Chinese institutions aiming to improve standards.

Since 2007 the RSPCA's International and Research Animal Departments have worked closely with government and academic institutions and laboratory animal science associations in both Europe and Asia to promote laboratory animal welfare, particularly ethical review and the 3Rs. This collaboration has included the sharing of materials, the provision of conference speakers and the delivery of training (Figure 1).

Figure 1. RSPCA workshop on laboratory animal welfare and the 3Rs, China
Photo credit: RSPCA

In 2007 RSPCA materials on the housing and care of 14 laboratory animal species were translated into Chinese by the Chinese Association of Laboratory Animal Sciences (CALAS) and were launched at the 3rd Congress of the Asian Federation for Laboratory Animal Sciences (AFLAS) in September 2008 in Beijing. At the same meeting staff gave presentations on ethics committees and the implementation of the 3Rs.

A two-day workshop held in Xi'an in 2009, focussing on good practice in laboratory animal care and the importance of sound experimental design in promoting the 3Rs, attracted around 90 CALAS members: scientists, academics, animal technologists and breeding establishment representatives. In 2010 a two-day course was given in Suzhou on 'Laboratory Animal Welfare and Alternatives'. Collaboration with CALAS continued in Yangzhou in 2012, with a presentation at the plenary of the association's annual meeting and in separate sessions on 'Refinement of Procedures' and an overview of laboratory animal welfare in the UK.

The RSPCA is currently funding a project, with support from NC3Rs, to develop with CALAS a Chinese-language version of the Procedures With Care website (www.procedureswithcare.org.uk/) produced by Newcastle University with the Institute of Animal Technology and NC3Rs. The Chinese site was launched early in 2014.

Action points:
- If you work for a company that has facilities overseas, including in China, find out whether these operate according to UK (or other good) standards with respect to animal housing, husbandry and care and ethical review.
- If they do not, consider how you could raise this issue and encourage 'levelling up', for example by seeing whether staff could be encouraged to join organisations like CALAS and to use resources such as the Chinese Procedures With Care site.

Aversion to rodent euthanasia agents

Huw Golledge, Newcastle University

Millions of laboratory rodents are killed for scientific purposes each year. This means that 'euthanasia' is effectively the most commonly performed technique in the laboratory, so it is critically important from both ethical and animal welfare aspects to ensure that it really does provide a 'good death' for the animals concerned. However, controversy continues to surround the question of which methods for killing rodents are the most humane.

Directive 2010/63/EU,[1] which regulates animal experimentation within the EU, requires that animals are killed with minimum pain, suffering and distress. It lists permitted methods for humanely killing animals, which in the case of rodents (excluding foetal and immature forms) are: physical methods (cervical dislocation, concussion and decapitation – the last of these only if no other method is 'possible'), anaesthetic overdose, a gradual chamber fill of carbon dioxide (CO_2) or inert gases (argon and nitrogen). Schedule 1 of the revised Animals (Scientific Procedures) Act (ASPA) governs the humane killing of laboratory animals in the UK but the UK chose not to include decapitation or inert gases in Schedule 1 due to welfare concerns about these methods.[2]

Concerns about CO_2

Although the techniques listed in Schedule 1 are generally believed to be humane, there are persistent concerns about some of them. For example, it is often suggested that the use of carbon dioxide may be inhumane since it can cause animals distress.[3] Nonetheless, it is still widely used, especially when there are large numbers of animals to kill. There are considerable incentives to use CO_2 as it is cheap, safe for the operator, easy to use, effective and does not contaminate tissues. Despite these benefits for humans, however, there are some animal welfare questions that deserve serious consideration:

- does CO_2 cause pain?
- does CO_2 cause distress?
- is anything else better?

Carbon dioxide is **painful** to inhale when the concentration reaches 50%, for humans[4] and probably for rodents too. This is because CO_2 forms carbonic acid when it comes into contact with water, which can occur on moist tissues such as the nasal passages, trachea and eyes. Being placed into a chamber pre-filled with CO_2 could therefore be extremely painful, which is why Schedule 1 stipulates that a gradual chamber fill should be used. Studies have shown that, with a flow rate of around 20% chamber volume per minute, rats and mice become anaesthetised before the concentration of CO_2 reaches painful levels.

Unfortunately, this does not solve the problem of **distress** associated with CO_2 exposure. Several studies have examined whether rats and mice find carbon dioxide *aversive*, i.e. whether they find it unpleasant to the extent that they actively avoid it and will become distressed if they cannot get away. This has been evaluated using 'approach-avoidance' tests, in which rats and mice are first trained to enter a test chamber for a highly desired food reward (e.g. Cheerios™ cereal) and once they have learned to enter this chamber for a reward, it is gradually filled with either the test agent or a flow of air as a control.[5,6]

When the incoming flow is just air, the animals will generally finish the food reward. However, when the flow contains CO_2, rats will leave at concentrations above 15%, even if there are still some rewards left.[6,7] Most studies like these have found aversion to relatively low levels of CO_2 in rats and mice – so although exposure to a gradual fill may not be painful, it is likely to be distressing. There is currently much debate about the level of distress caused by CO_2 and whether it is such that CO_2 killing is not humane.

Is anything **better**? Anaesthesia with isoflurane prior to killing with CO_2 (or argon, or another non-inhalation method) has been suggested to be a more 'humane' alternative, yet it is unclear whether isoflurane causes less aversion than CO_2. Approach-avoidance tests based on a single exposure to the gases, as above, have shown that isoflurane is less aversive than carbon dioxide[8] and argon is considerably more aversive than either of the other two agents.[6] It looked as though there was a simple scale of degrees of aversion, but more recent research has complicated this. On the basis of the mouse or rat's first exposure to isoflurane, it is less aversive than CO_2 – but on second and subsequent exposures, it is equally aversive to rats[9] and possibly to mice, although this has yet to be tested. Thus, aversion to isoflurane appears to be at least partially learned. This has implications for studies that involve anaesthetising animals multiple times such as repeated imaging studies, and also calls into question whether anaesthesia before switching to CO_2 is truly a refinement for animals who have already been exposed to isoflurane. (NB other studies have found that the learned aversion persists for some time and is transferred to other volatile agents such as sevoflurane.)

Ongoing research and new techniques

There have been some criticisms of the conclusions of studies involving exposing animals to agents and measuring aversion. For example, an animal might leave the chamber when the concentration of the test agent reaches a certain level but does that mean it would cause significant distress if the animal was

unable to leave? Is the gas really aversive, or does it simply alter motivation or make the reward less palatable?

A technique that can be used to address these questions is 'conditioned place aversion', which measures animals' abilities to associate the environment where they have been exposed to an agent with their response to that agent. If the exposure is aversive (e.g. if a gas is unpleasant to inhale), then the animal should learn to avoid the environment they associate with that exposure. This is an especially convincing technique because it uses the animals' memory of what has happened to them, rather than the cues they experience at the time, as the animal avoids the environment even when the agent is not present. This is regarded as an indication of the affective, or emotional, state of the animal induced at the time of exposure.

A typical conditioned place aversion protocol presents animals with a choice between two chambers; one with plain walls and a smooth floor and the other with distinguishing features such as striped walls and a textured floor (Figure 2). Each animal is trained to expect a flow of air in one chamber and a flow of the test agent in the other by confining them in each chamber during training sessions. Once training is complete, the animal is placed into the apparatus with just air in both sides and allowed to move freely between the two chambers and the amount of time they choose to spend in each chamber is recorded. If the test agent is aversive, the animal should decide to spend significantly less time in the chamber that they have learned to associate with the agent.

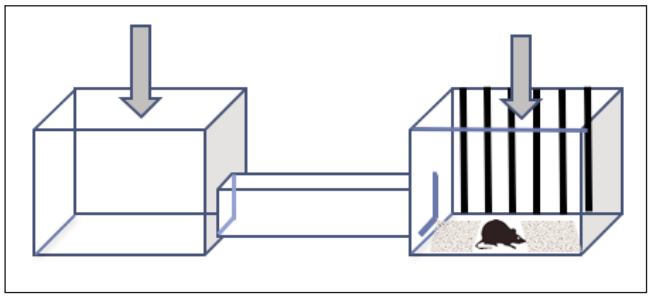

Figure 2. A typical 'conditioned place aversion' experimental set up

The rat is trained to expect a flow of test agent in the left hand (plain) chamber and a flow of air in the right hand chamber, which has patterned walls and a textured floor. Following training, the animal is placed in the chamber with air in both sides and their position is monitored (see text).

We have been using this protocol, in research funded by the NC3Rs and UFAW, to evaluate the comparative aversiveness of carbon dioxide, isoflurane and argon. Studies so far indicate that all three cause significant place aversion. Argon is also significantly more aversive than CO_2, confirming that the UK made the right decision when excluding inert gases from Schedule 1. However, care is needed when interpreting the results for isoflurane and carbon dioxide. These are both apparently equally aversive but repeated exposures were necessary to train the animals – so aversion to isoflurane could have been learned, as outlined above. This means that the aversiveness of isoflurane for naïve animals, relative to CO_2, could be overestimated using this technique.

What causes aversion to CO_2?

All of the above research raises the question of what actually causes the aversion that is displayed towards all three agents by mice and rats. This is easiest to explain for CO_2. Rats and mice are both burrowing species, so their sensitivity to carbon dioxide is likely an adaptation to ensure that they can sense and be motivated to escape from pockets of accumulated CO_2. In fact, mice can smell and will avoid CO_2 at concentrations of just 0.2%, which is little above atmospheric levels.[10] Breathing low levels of CO_2 causes feelings of anxiety in humans and brief exposure to 20% CO_2 also activates 'panic/defence' brain circuitry in rats[11] via direct detection (in the amygdala) of the acidosis caused by inhaling carbon dioxide.[12] The reason for the very marked aversion to argon is unknown but may relate to a heightened response to hypoxia or asphyxia. In the case of the learned aversion to isoflurane, it has been hypothesised that animals remember feeling nauseous when recovering, which motivates them to escape when they smell the agent again. The implications for repeatedly anaesthetising animals with gaseous agents also need to be further explored.

To conclude, research to date suggests that no inhalation agent so far tested is likely to offer an entirely humane method for killing laboratory rodents. Carbon dioxide is not ideal but it is unclear whether isoflurane is significantly better – and inert gases are almost certainly worse. A continued search for a humane method is required.

Note: The second Newcastle Consensus Meeting on euthanasia was held in August 2013 and the forthcoming report (in preparation at the time of writing) will address the above issues in more depth, along with physical methods and euthanasia of fish.

Action points:

- If CO_2 is used to humanely kill rodents at your establishment, suggest that practice is reviewed, taking the current literature into account. This could be initiated by the Named Information Officer (NIO), or Named Animal Care and Welfare Officer (NACWO), reviewed by the AWERB, or discussed more widely.
- Ensure that the AWERB regularly reviews the literature on humane killing in general, so that good practice is maintained in line with current thinking.

– Watch out for the publication of the second Newcastle report and bring it to the attention of your NIO.

Assessing welfare in mouse disease models

Claire Richardson, Newcastle University

A key component in reducing suffering within disease studies is the implementation of appropriate humane endpoints. Humane endpoints can be defined as 'criteria that allow early termination of experiments before animals experience significant harm, whilst still meeting the experimental objectives' (see http://www.humane-endpoints.info). The effective implementation of humane endpoints therefore depends upon recognising when the experimental objectives have been met, unless severity limits are approached or exceeded.

Today it is thankfully widely accepted that death is not an acceptable endpoint. Ideally, humane endpoints should not be considered as predictors of imminent death, but rather as ways of identifying animals in more 'moderate' clinical states so that appropriate action can be taken to ameliorate suffering.

However, the use of animals as disease 'models' presents a problem with respect to refining and implementing humane endpoints. It may be necessary to allow animals to become sick in order to answer the experimental question and this may cause pain and distress as the disease progresses. But it is possible to set limits on disease progression and this is not only essential from an animal welfare aspect but will also minimise experimental variability, leading to reductions in numbers.

Achieving these limits obviously requires an understanding of how far disease has progressed in each animal. However, different disease 'models' have different effects, such as pain, malaise, nausea or fatigue, some of which are difficult to detect and assess. It is essential that observations are made by experienced, compassionate staff, using carefully considered indicators and record keeping systems. Work is also in progress to identify 'surrogate markers' of disease progression, as a way of further refining both scientific and humane endpoints.

Imaging is frequently advocated as a method of tracking disease progression but repeated general anaesthesia is typically required which may affect experimental outcomes and have welfare implications[9,13] (see also Golledge, this paper). Additionally, imaging is expensive, requires specialist skills and equipment and may not be carried out at a sufficient frequency to detect critical points in disease progression. We therefore need to identify non-invasive biomarkers (e.g. subtle behaviours or clinical signs) that can be used as specific indicators of disease progression.[14,15] Beyond their importance in humane endpoints, biomarkers that reliably predict the onset of clinical signs can also be used to introduce refinements at times when they are most needed by the animals.

We are developing an enhanced clinical monitoring approach, funded by UFAW and the NC3Rs, using minimally invasive radiofrequency identification (RFID) technology, to try to define useful biomarkers to help track disease progression. RFID chips, which transmit body temperature, are implanted into mice already involved in disease studies, so that data can be obtained without creating disease models just for that purpose and without causing any additional suffering apart from implanting the chip.

So far, this technology has been used to monitor temperature in mice used in lymphoma studies. In this procedure, tumour cells infiltrate the lymphoid organs and the mice develop enlarged lymph nodes, spleens and thymus glands but the tumour burden cannot be measured directly. Food and water consumption decrease as tumours grow and mice develop clinical signs of sickness including hunching, huddling together, 'staring' coats, dull demeanour and ears pulled back from baseline position. A decrease in mean body temperature was then found to predict tumour burden effectively in these mice.[16]

As another example, Radio Frequency Identification (RFID) chips were implanted into mice used in a study to develop therapies for liver fibrosis which involved bile duct ligation. This is a severe procedure and several measures are in place to refine it to reduce suffering, such as providing heated areas in the cage and special attention to perioperative care and pain relief. In addition to the body temperature data, transponder technology was also used to monitor individual water consumption. In this case, body temperature did not correlate with the level of liver fibrosis but drinking bout duration did, as this was significantly reduced in animals with fibrosis.

The use of enhanced clinical monitoring to identify biomarkers to help track disease progression therefore shows considerable promise, provided that the most relevant biomarkers are identified for each model and experimental question.

Action points:
– Question and challenge the use of endpoints that involve animals suffering from advanced disease states.
– Ensure that indicators of pain, suffering or distress are tailored to each disease study and can detect subtle signs of suffering.

– Keep up with current information on new techniques and approaches to monitoring animals, using these to detect suffering and refine humane endpoints.

Refining rheumatoid arthritis – a 'joint' approach

Sarah Allden and Tania Boden, UCB

Collagen Induced Arthritis (CIA), Glucose-6-Phosphate Isomerase (G6PI) arthritis and Collagen Antibody Induced Arthritis (CAIA) are all polyarthritis 'models' that are commonly induced in mice. However, these models differ with respect to the way they are induced, their speed of onset and the severity of the arthritis, suggesting that 'generic' approaches to welfare assessment and refinement would not be appropriate. The introduction of the G6PI and CAIA models therefore prompted a re-evaluation of existing welfare scoring sheets, alongside husbandry refinements such as improvements to environmental enrichment, in order to improve the overall welfare of animals used in arthritis studies at our facility.

The original record sheet we used for assessing CIA mice was based on Wolfensohn and Lloyd,[17] with scores reflecting progression in paw inflammation. This was useful for the CIA mice but it soon became apparent that it would have to be tailored for the G6PI and CAIA models. This triggered discussions involving scientists, animal technologists and the NACWO, to determine how best to go about modifying the way in which these animals should be assessed and monitored. Good communication between all and consensus with respect to indicators and the terminology used to describe them, helped us to make significant refinements to both our monitoring systems and procedures.

To begin with, the mice used in G6PI and CAIA studies were very closely observed by both researchers and care staff, particularly with respect to weight loss and disease progression. We collated the data to assess how many individuals lost weight below a certain criterion and how many had a maximum disease score, in order to see how we might reduce the number of animals approaching these levels. Proactive interventions were established whenever the need arose, for example weight loss was reduced by diet supplementation and humane endpoints were defined to prevent severe arthritis.

The observations were used to create a scoring system, using a standardised terminology for describing and logging observations, which was specifically tailored to each model. Some indicators were common to all models, such as weight loss, appearance (coat condition) and behaviour (isolation from cage mates). The humane endpoint for weight loss was refined by reducing the maximum from 25% to 20% and including another endpoint of a 15% weight loss that had not begun to reverse after 5 days.

Other indicators varied between models. For CAIA, some indicators that occurred in the short term following the administration of Lipopolysaccharides (LPS) were added; soft stools, ocular and nasal discharge. Problems with mobility and dehydration were added for G6PI. The use of tailored indicators for each model also enabled us to reduce the length of time that animals spent on procedures, thereby reducing suffering; for example, the CIA studies were successfully reduced from 30 days to 20. The benefits of the tailored scoring systems are summarised in Table 1 below.

	Old sheets	New sheets
Model specificity	No	Yes
Cumulative suffering	Not addressed	Maximum scores over time taken into consideration
Weight loss	Endpoint of 25% or 20% over 72 hours	Endpoint of 20% or 15% over 5 days
Adverse events	No specific details	Model specific observations on sheets
Individual disease score	Total disease score not taken into account. Distress scoring based on number of swollen paws, not severity	Total mouse disease score captures severity more effectively

Table 1. Summary of distress sheet refinements in polyarthritis studies

Alongside the work to refine welfare assessment, we have worked to develop the environmental enrichment provided for the animals. DBA/1 male mice are used in the CIA studies and territorial behaviour was observed when a refuge was provided in the cage. We have found that simply adding another house has solved the problem, as the dominant mouse takes possession of one house and the rest of the group uses the other one. One of the houses has an integral running wheel, to encourage activity before and sometimes after disease induction. Different kinds of nesting material have been trialled, to see which one stimulates nest

making and is also best for inflamed paws – paper shavings are now provided because they are less likely to tangle than wool shavings.

When mobility becomes restricted during the acute phase of the disease, longer nozzles are added to the water bottles so that animals do not have to rear up and place additional pressure on their hind limbs. Food is also made accessible around the cage, including supplementary diets to reduce weight loss, especially following administration of LPS.

Refinement is a continuous process and we are always seeking further modifications. For example, we have begun to use the Mouse Grimace Scale[18] for welfare assessment and refining endpoints and we are introducing non-disease modifying pain management in some of our studies. All of these factors combine to result in a proactive approach to better animal welfare – and improved experimental results.

Note: as part of our work on reducing severe suffering, the RSPCA has set up an Expert Working Group on applying the 3Rs in rheumatoid arthritis research using mice and rats. Tania is a member of the group and a report is currently being produced – if you would like updates on progress with this, contact **research.animals@rspca.org.uk**

Action points:
- Consider whether there is a similar 'joint approach' to welfare assessment and refinement at your establishment. If not, how could you encourage this?
- Review how effectively welfare assessment protocols are tailored to species, strains and procedures at your establishment.
- If you are involved with using or caring for animals in arthritis research, consider some of the husbandry refinements in this section – and contact the RSPCA to express an interest in the forthcoming report.

Are behavioural scientists aware of the natural history of the animals they work with and does it matter if they're not?

Colin Hendrie, University of Leeds

Much attention is given to the physical conditions in which laboratory animals are housed, including environmental enrichment but very little attention is given to meeting their social needs. There are compelling animal welfare and scientific reasons for understanding and catering for the behaviour of laboratory animals, including rodents.

In the case of commonly-used rodents, rats are a colonial species and both sexes generally do well when housed in groups. Mice, on the other hand, are highly territorial and this is reflected in the fighting that is commonly seen when males are group housed. In a typical cage of group housed male mice, one individual will become dominant with the rest being subordinates. If group housed male mice are exposed to inescapable aggression, this will cause severe suffering. Besides these welfare problems, animals of each social rank display different behavioural, physiological and immunological profiles, which will inevitably increase the variance in any experiment and can even render the results meaningless.[19]

Understanding the social behaviour of rodents should therefore be a top priority for any scientist using these animals in research and testing, but it is not. A meta-analysis of 100 research papers using mice and rats, published in 2010-11, found that 99 of these failed to take into account the social organisation of these animals – and the one that did, got it wrong.[20]

A further online survey was conducted to explore the level of knowledge that behavioural researchers possess about their study species. This asked questions about the biology and behaviour of a range of species including the fox, rat, mouse, elephant and tree kangaroo (the tree kangaroo was included to detect respondents who looked up the answers, on the assumption that few people are even aware these animals exist). Answers from behavioural scientists working with laboratory animals were analysed and compared with those from scientists working in other areas and members of the public. The study found that the behavioural scientists knew very little about the animals they worked with and had no specialist knowledge of these animals, beyond being familiar with their Latin names and some trivial physical characteristics such as adult weight and longevity.

These findings have implications for scientific quality and translatability. In particular, lines of GA mice are created using the behaviour of 'standard' laboratory mice as the 'wild type' – with little or no knowledge of the behaviour, social organisation or natural history of the wild mice from which these laboratory strains were derived. This leads to misinterpretations of the behaviour of GA lines; there are many examples in the literature where normal mouse behaviour has been mistaken for the effects of genetic alteration. Examples of this are male mice fighting when one animal is introduced into the home cage of another and species- and strain-specific variations in responses in common models of anxiety such as the elevated-plus maze. Although it is commonly assumed that inbred or GA laboratory strains are behaviourally far removed from their wild types, 'natural' behaviours are in fact strongly conserved and innate. This is effectively demonstrated by the 'Ratlife' documentary in which laboratory rats

were released into a semi-wild enclosure (see www.ratlife.org) and the ability of laboratory mice to recognise the calls of murine predators.[21]

The above indicate that there is a major educational task ahead. This needs to encompass initial training and Continuing Professional Development for researchers. Available training and resources should be complemented with input from animal technologists, who have expertise in animal biology and behaviour that could help with the interpretation of behavioural tests and also with experimental design. However, achieving this depends on effective communication and collaboration and appropriate status afforded to animal technologists and care staff.

Action points:
- Suggest discussion of this issue at your establishment, including general awareness of the importance of understanding behaviour and review of whether there is sufficient communication between people with different expertise, including researchers, animal technologists, veterinarians and the NIO.
- If you are a **researcher** – consider how familiar you are with the behaviour and biology of your study species. Would you like more training and if so would this be readily accessible?
- If you are an **animal technologist** – consider whether you are satisfied with the training and CPD that you have received/do receive with respect to animal behaviour and natural history. Would you like more training and if so, would this be readily accessible?

Welfare assessment of Genetically Altered (GA) rodent lines: what is out there?

Dominic Wells, Royal Veterinary College

There are a number of reasons for assessing the welfare of GA rodent lines. In common with 'conventional' strains, effective welfare assessment allows the prompt identification and alleviation of any health or welfare problems, which gives rise to both scientific and animal welfare benefits. There is also a legal requirement under the revised ASPA for the actual severity experienced by each animal to be retrospectively assessed and reported. A further reason, specific to GA animal use, is the acquisition of information relevant to maximising welfare that can be passed on to others, for example as part of the GA passport. [22]

Genetic alteration has the potential to cause suffering if the GA line is a 'model' of a disease that causes pain or distress; if the gene disruption leads to a physical impairment; or if there is an unexpected adverse effect of a random integration or a targeted gene disruption. As a result, welfare assessment of GA rodents can raise some specific issues over and above observing and monitoring 'conventional' lines. For example, it may be difficult (or impossible) to predict adverse phenotypes and any welfare impact these may have on the animal and some adverse effects may not become apparent until certain developmental stages.

In the early days of GA rodent creation and use in the early to mid 1980s, there was a 'wait and see' approach to welfare assessment, in which records of increased morbidity and mortality were simply analysed retrospectively. Practice has moved on considerably since then and a number of studies have examined the welfare of GA mice.[23-26] Score sheets for assessing and monitoring the welfare of GA mice were proposed by Mertens and Rülicke[27] and van der Meer et al.[28] and a 2003 review of the literature by Jegstrup and colleagues concluded that there was a clear need to develop a generally applicable and practical protocol.[29]

Similar conclusions had also been reached by the Animal Procedures Committee (APC), the independent body that advised the Secretary of State on the implementation of the ASPA until the end of 2012 (when it was superseded by the Animals in Science Committee). The APC produced a report on biotechnology in 2001, making a number of recommendations relating to the welfare assessment of GA animals, particularly mice. In response, major UK research funders* established an expert working group to define a flexible protocol for welfare assessment.[30] This suggests appropriate welfare indicators and reporting formats, emphasising the need for comprehensive assessments of each new line, from neonates to adults. These assessments should be revised whenever breeding onto a different genetic background, breeding to homozygosity or crossing with another GA line.

The 2011 Joint Working Group on Refinement guide to welfare assessment[31] provides additional guidance, intended for those responsible for assessing both 'conventional' and GA animals. It emphasises the importance of a team approach, with input from researchers, animal technologists and veterinarians, plus input from (or discussion with) local ethical or animal care and use committees such as the UK AWERB if appropriate. A longer version of the guidance includes recommendations on training and a list of further resources (see also www.nc3rs.org.uk/welfareassessment for some useful links).

* The Biotechnology and Biological Sciences Research Council (BBSRC), Cancer Research UK, Medical Research Council and Wellcome Trust.

Most recently, the European Commission has produced some guidance on welfare assessment, including actual severity assessment,[32] with some worked examples including GA animals.[33] The Home Office has also produced advice notes on severity assessment of GA animals and on actual severity reporting.[2] However, there are currently still some areas where more clarity is needed with respect to classifying actual severity in GA mice. These include:

- distinguishing between 'mild' and 'subthreshold' severity
- differentiating between knowledge of a GA line (i.e. what is expected, or what is known at a cellular level) and clinical observations of the animals
- how to classify the severity of a 'sudden death' e.g. in GA models of some cardiomyopathies.

Action points:

– Ensure that the literature on welfare assessment, especially with respect to GA animals, is regularly reviewed and its approaches and recommendations implemented (where appropriate) at your establishment.

How and where to start with GA severity assessment

Nikki Osborne, RSPCA Research Animals Department

Article 54 of Directive 2010/63/EU requires Member States to collect and report statistical information on the *actual* severity of the pain, suffering, distress or lasting harm experienced by each animal. To help Member States comply with what was (for most) a new requirement, the European Commission held two expert working group meetings on severity assessment in 2012. The first considered the specific severity issues related to the creation, production and maintenance of GA animals*, while the second addressed severity assessment more generally. These meetings resulted in the *Working Document on a Severity Assessment Framework* that was endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU in January 2013.[32]

On the 9th September 2013, the Wellcome Trust Sanger Institute hosted a meeting on 'Severity Assessment and Actual Severity in GA Animals'. This was attended by a range of staff (including managers, animal technologists and Named Persons) from research establishments using GA animals in the Cambridgeshire and Oxfordshire area, as well as representatives of the Home Office Animals in Science Regulation Unit. Some, but not all, of the represented establishments took part in the Home Office pilot study of new statistical reporting standards and draft guidelines, which ran from August to October 2013. A full report of the meeting is currently being prepared; the summary points below represent an overview of some of the meeting outcomes.

The **reasons** for assessing actual suffering were understood; '*…inclusion of the actual suffering experienced by the animal provides greater transparency and understanding of the impact of scientific procedures on animal welfare.*' This is not an additional 'burden' but takes the observations that animal technologists already make on a daily basis and uses them to inform the severity assessment – in doing so, their expertise in knowing what is normal and what is not normal, for the animals in their care will be acknowledged and contribute to improving animal welfare.

– **Severity assessment encompasses**:
  - identifying when an animal is experiencing pain, suffering, distress or lasting harm
  - recording whatever it is that is 'not quite right' and
  - deciding how severely the identified animal is suffering.

Anyone who believes that an animal is experiencing pain, suffering, distress or lasting harm should make a record of it.

– **Signs of pain, suffering, distress or lasting harm are currently detected**:
  - during routine husbandry and care procedures (e.g. cage change, identification)
  - during experimental procedures
  - during routine observations, or welfare assessment checks or
  - during phenotyping procedures.

Generally all animals are observed for some reason, at some point, once a day (see Hutchison, this paper).

– **Observations are commonly noted** by:
  - discussing with a colleague/NACWO/NVS
  - entering them either physically or electronically into a structured database or spreadsheet
  - writing them down and filing them
  - writing them down, and storing them somewhere with communal access or
  - noting and discussing them with the project licence holder, or NVS.

The important point is that all observations should be recorded and kept in some traceable and reviewable way.

* NB The Statistical Environment Reporting Framework for Directive 2010/63/EU states that *'animals from genetically altered lines include transgenic, knock-out and naturally occurring or induced mutant animals, and other forms of genetic alteration, regardless of phenotype'.*

– **Everything that is 'not right' should be recorded as seen** – but this does not involve making a diagnosis. For example, observations may include: reluctance to be handled, closed eye, wound on tail, unsteady walking/gait, nasal discharge, reduced food/water intake, not interacting with cage mates. To enable consistency, records should use a controlled vocabulary, see www.mousewelfareterms.org. This will also enable trends to be identified over time and data to be reviewed or analysed in an informative way.

– **Observations and clinical signs** that could be used to assess severity are listed in Table 2. These have been sorted into the 'high level categories' set out in the EC guidance.[32]

'High level' categories	Commonly used indicators for GA rodents
Appearance	Weight, body condition, general appearance, coat (e.g. piloerection), discharges (e.g. ocular, nasal, mouth, urogenital), masses or growths, pallor, facial expressions
Body functions	Respiration, food and fluid consumption, cold to touch, moribund, breeding related indicators (e.g. parturition), neurological signs, grip response
Environment	Presence, location and consistency of faeces and urine
Behaviours	Mobility, vocalisation (audible), socialisation (responses to cage mates and humans), convulsions, mutilation (of self or others), provoked behaviours, changes in response to environment, writhing, hunching, lethargy
Procedure-specific indicators	Death, pre-weaning mortality
Free observations	There should always be a facility to note any other observations that may be unexpected or impact on animal welfare/suffering

Table 2. Examples of commonly used welfare indicators for GA rodents

– Clear, GA relevant definitions were suggested by the delegates attending the Wellcome Trust meeting in September for use when assigning severity classifications:
  – Sub-threshold – looks and behaves like a 'wild-type' mouse and has the same housing and husbandry requirements.
  – Mild – housing and husbandry needs to be adapted to maintain the mice in a 'normal' condition, e.g. they may require IVCs, adapted feeding methods, special diets or supplementary feeding or adjustments to environmental temperature.

– **Legal requirements and points of guidance were clearly understood by the group:**
  1. The actual severity that should be reported annually to the Home Office, as of 2014, is the peak severity that an individual animal has experienced; e.g. if an animal experiences mild severity which then drops back below threshold, the actual severity following the end of the procedure should be reported as 'mild'.
  2. If an animal is identified as having exceeded the severity limit of the protocol, this must be reported to your HOI at the earliest opportunity (with the understanding that actual severity assessment will not occur until after the procedure has ended).
  3. If an animal is 'found dead', actual severity should be classified as 'severe' unless there is evidence to the contrary.

– **Other aspects were less clear**, with some outstanding questions:
  1. If there is a 2% spontaneous death rate in the background strain and a 4% death rate in the GA line, all animals found dead must be counted – it is not permissible to discount the first 2%, but can (and if so, should) 'background' effects be taken into account?
  2. Would repeated mild procedures constitute moderate suffering – if so, when and how should cumulative effects be taken into account?
  3. Is it possible (and is there a need) to distinguish between husbandry and procedure-related effects?

Action points:
– Discuss the summary points with colleagues, including the 'difficult' questions under the last bullet point. This can start, or further inform, the process at your establishment with respect to reporting actual severity.
– Consider whether your establishment has all the necessary protocols in place to assess severity affectively in GA animals. This includes both day-to-day welfare assessments and the assessment of actual severity after the procedure has finished.
– If your establishment does not already use www.mousewelfareterms.org, suggest that it does.

The importance of welfare assessment in an ageing rodent programme

Marie Hutchison, Mary Lyon Centre, MRC Harwell

A major aim of the ageing programme at MRC Harwell is to produce mouse mutants that can be used to study diseases that affect ageing human populations. This is because age-related diseases are becoming increasingly common which is putting pressure on society as well as compromising the health and wellbeing of those who are directly affected. Large cohorts of ageing wild type mice are housed at Harwell, with over 5,000 aged mice in the current screening programme. These animals can live for 18 months, so effective welfare assessment is essential to detect signs of suffering due to an animal's age or other adverse phenotype.

Basic, yet thorough, routine cage-side observations are essential when looking for any abnormalities or welfare concerns in ageing animals. Significant amounts of time are allocated for daily observations of animals, to check their behaviour and appearance, with open-cage checks for closer inspection as needed. Comprehensive in-house training and familiarity with the normal appearance and behaviour of a healthy animal are especially important, helping to ensure that even very subtle differences can be recognised and monitored from the time they appear.

A full 'nose-to-tail inspection' is carried out of each animal, including an examination of head shape, facial features, limbs, behaviour and activity, body shape, digits, tail length, coat condition and colour. This assessment is carried out in addition to standard phenotyping protocols and is done at different time points; at 4 weeks of age, then every 6 weeks for animals under 16 weeks or over 1 year old, or every 12 weeks for animals aged between 16 and 52 weeks. Standardised wording is used to describe observations (as in www.mousewelfareterms.org) to ensure consistency and continuity. These observations and the accurate records that accompany them, enable more subtle details to be picked up, which has been crucial in the discovery of new phenotypes and the refinement of humane endpoints.

All mice exhibiting an adverse phenotype or any other welfare concern, are recorded and examined throughout their lives to monitor any progression or degeneration in condition. Weighing regimes are an informative tool in assessing the health of an individual animal and frequent weight data collection is an essential element in assessing if an animal's health is deteriorating. However, a degree of weight loss can be normal in some strains as the animals age or a line may be lean but healthy. Weight should therefore be interpreted in conjunction with other indicators, although rapid weight loss is always a cause for concern. For most strains, animals are weighed according to the same time points as the morphology check above. Frequency increases to weekly if a loss of 8% or more is noted, daily if a loss of over 15% is noted, and animals are humanely killed if more weight is lost or if there are other complications. In addition to increased weighing frequency, mice with weight loss are palpated for growths and undergo a full welfare assessment.

Assessing the welfare of aged animals comes with some challenges – we often have to consider carefully whether an animal is sick or just old but otherwise healthy. For example, hair loss and thinning is observed in ageing mice (Figure 3). Elderly animals are also prone to benign lumps and swellings under the skin (these are carefully monitored) and lesions sometimes appear, which are treated with a topical cream for three days, after which animals are humanely killed if there is no improvement. Some dominance behaviours, such as barbering, can also become more marked in the long term and offenders may have to be separated from the group and housed individually for short periods.

Figure 3. Normal hair loss in a healthy ageing mouse
Photo credit: MRC Harwell

The data that we gather, through careful open-cage checks and daily observations, have all contributed to establishing an effective routine of cage-side welfare assessment and phenotyping. Some phenotypes require very careful welfare assessment in order to identify when an animal has reached the humane endpoint for the particular line. For example, unexpected mortality was observed in one line, with no obvious predictors, which was a serious concern. However, with enhanced monitoring, it was found that gait changes and continuous eye blinking occurred before death, which has enabled humane endpoints to be defined and mortality to be reduced. The phenotype is still under investigation but is thought to include a cardiac or neurological disorder. As another example, careful daily monitoring of the weight of another line with higher mortality than expected has enabled

humane endpoints to be defined, so that the severity limit has been reduced from severe to moderate. This line has a renal phenotype.

The above approach has reduced the effects of adverse phenotypes experienced by the mice and it is hoped that the aged lines of mice will be of use in studying human disease. Animal technologists should not undervalue the role they play in assessing the welfare of animals, as their key observational skills and the ability to pass this information on to other research and technical staff is essential in helping to ensure that the animals' welfare requirements are met.

Action points:
- If you use or care for ageing rodents, set a target for reducing mortality levels by identifying new predictors of death and refining humane endpoints.
- Make sure that your group is in contact with others using aged animals, to exchange ideas and information about monitoring animals, reducing suffering and improving welfare.

Actual severity assessment and retrospective reporting

Steve Ryder, Home Office Inspectorate

As mentioned earlier, Directive 2010/63/EU requires Member States to collect and report statistical information on the *actual* severity of the pain, suffering, distress or lasting harm experienced by each animal. This has been transcribed into the revised ASPA, which came into effect in January 2013 and resulted in some changes to the way in which the annual Return of Procedures is collected and reported (see ASPA Sec 21A(1)2(a))[2].

The major changes are:

1 procedures are counted once completed, as opposed to when started;
2 the actual severity of all procedures must be assessed and recorded after the end of the procedure.

Assessing actual severity is a requirement for the annual Home Office Return of Procedures and is also a prerequisite if the project licence permits re-use, as there are restrictions relating to the severity of previous procedures.

As of 1 January 2013, the Project Licence Holder is responsible for ensuring that the actual severity of procedures ending during 2014 is assessed and collated for submission to the Home Office. It is essential to be aware that actual severity must be judged from the day-to-day records of each animal's health and welfare, not the prospective severity of the protocol. It should reflect the worst experience of each individual, in terms of the impact on the animal and not the technique applied. In practice, the prospective and actual severity may be the same – but this will not necessarily always be the case.

Actual severity will be reported according to the same categories as those used for prospective severity classification; mild, moderate, severe and non-recovery; but there will also be a 'subthreshold' category, in which the actual severity experienced by the animal was below the threshold for regulation ('*a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice*'). As a hypothetical example, animals were given an altered diet and some were also given an intraperitoneal (ip) injection of a drug, where neither the drug nor the diet was expected to be harmful. The prospective limit was mild. The altered diet was found not to cause any adverse effects, so the actual severity for those animals only receiving the diet was subthreshold. The animals receiving the intraperitoneal injection experienced transient discomfort but the drug had no adverse effects, so their actual severity was mild.

The Guidance on the Operation of ASPA includes sections on defining severity categories and the classification of actual severity is addressed in more detail in a separate advice document.[2] Some difficult issues were identified during the drafting process; how to take account of procedural and non-procedural effects; how different species and stages of development experience suffering; 'found deads'; cumulative suffering – and GA animals. Some special issues that arise with GA rodents include neonatal deaths and how these should be classified, poor fertility, late weaning and innate morbidity or mortality in background strains. Genotyping methods and some phenotyping protocols, e.g. SHIRPA, also have the potential to cause discomfort or distress, thus adding to cumulative suffering.

The UK currently regards 'breeding' of GA animals as a regulated procedure, with most currently licensed with a mild severity limit. At present, virtually all breeding of GA animals is within the regulatory process. In future however, only the breeding of lines with phenotypes that are above threshold should be regulated, which will likely require some rethinking of the definition of 'mild' for GA animals.

Article 30 of the Directive requires that records are kept of the number and species of animals bred but not used in regulated procedures. These should be kept for at least 5 years and made available to the competent authority on request. The Home Office proposes to collect these data either annually, or relating to one year in every five, commencing in 2017. GA animals will be distinguished from wild type, providing an indication

of the number of animals humanely killed because they do not have the required genotype or are surplus to requirements.

The Home Office Guidance to the revised ASPA and the advice document on severity assessment, are both now complete and published on the Home Office website.[2] There will also be the ability to revise and update the Guidance and advice document as required – see https://www.gov.uk/research-and-testing-using-animals for further information and to check the current status of these documents and other relevant guidance and advice notes.

Action points:

- Ensure that you are familiar with the guidance on assessing and reporting actual severity.
- Ask your Home Office Inspector if you are unsure about how to categorise actual severity in any 'difficult' cases.
- Remember that actual severity assessment should provide information to help progress refinement and reduce suffering; it is not just about the statistics.
- Consider what role the AWERB might play, or what kind of oversight it will have, regarding assessing actual severity.

Interactive session – assessing actual severity in GA mice

Steve Ryder and Penny Hawkins

Following on from the presentations on assessing GA animals, an interactive 'TurningPoint' session was used to find out more about current practice at delegates' establishments and provide some hypothetical examples of adverse effects that could be observed in a breeding colony of GA mice. An overview of the outcome is presented below; note that the numbers of responses vary according to the number of people who voted or abstained, or whether more than one option could be selected. These are included in this report as an example of the delegates' views on the day, to stimulate thought and discussion; for guidance on actual severity assessment please refer to the Home Office.

Most of the 90 delegates were animal technologists (38 votes), NACWOs (11) or researchers/students (7). The remainder included other Named Persons (Named Training and Competency Officers, Named Information Officers and Named Veterinary Surgeons, establishment licence holders), with one Home Office Liaison Officer and three regulators (it was possible to vote for more than one role). The majority worked at an academic establishment (22 votes), Government agency (13) or medical or veterinary research institute (14), with fewer delegates from industry, commercial companies and welfare organisations. Most delegates who voted worked at establishments housing at least 1,000 GA animals (Figure 4).

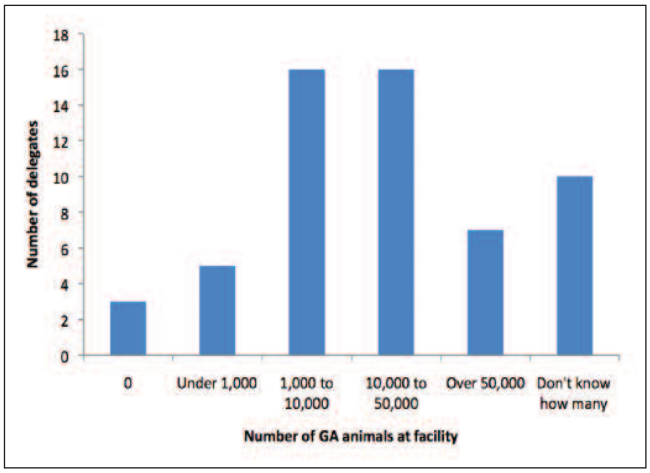

Figure 4. Number of GA animals housed at delegates' facilities

Routine welfare checks were generally carried out once a day (60% of the vote) or twice a day (22%). Observations were recorded whenever they were made (25%), or whenever there was something to record (60%), in most cases. In some instances (7.5%) records were only made when action was required. Records were kept on paper (42 votes), and/or electronically (39), and/or verbally (23). Two delegates responded that observations were not recorded.

Not all GA animals were reported to undergo specific, formal welfare assessment at the cage-side, although most did if there appeared to be a welfare problem or there was some scientific interest (Figure 5). With respect to the actual severity assessment following the end of the procedure, five delegates voted that this should be done by the researcher only. In contrast, 39 voted that the judgement on actual severity should be

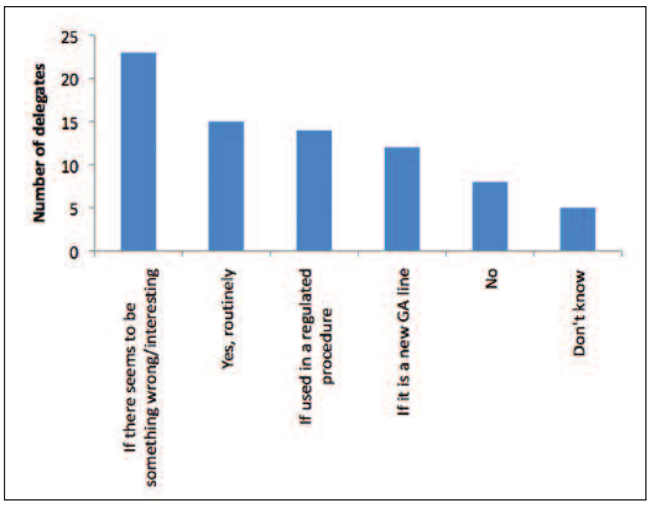

Figure 5. Responses to the question: 'Do GA animals undergo specific, formal welfare assessment in addition to phenotyping'

made by the researcher with input from others. Animal technologists (35 votes) and vets (37) received the most votes as those who should help to make the decision. Study Directors received 14 votes and the AWERB 12, while eight delegates agreed most with the statement 'it depends'.

The rest of the interactive session was devoted to discussion of some hypothetical, but realistic, cases relating to the breeding of GA mice (Tables 3 and 4).

	Below threshold	Mild	Moderate	Severe
The offspring of a GA mouse who is weaned normally, has no observable adverse phenotype, is ear notched for genotyping and killed by a Schedule 1 method as 'surplus stock'	15	44	0	0
A nude mouse ear notched for identification, and maintained within a barrier facility with no evidence of illness before being killed by a Schedule 1 method at 3 months old	24	21	8	0
A GA mouse ear notched for identification, from a line that develops spontaneous tumours – but who is killed by a Schedule 1 method at 8 weeks old, before tumours develop	34	17	5	0
A GA mouse who dies as a result of a one-off, spontaneous seizure	1	2	11	34

Table 3. Delegates' views on actual severity in relation to the breeding and husbandry of GA mice

Situation	Number of delegates voting that actual severity was 'severe'
2 year old breeding stock found dead	27
A GA mouse displaying almost continuous circling behaviour, from which they cannot be distracted	25
Death of a GA mouse due to facility infection with MHV (that did not kill any non-GA mice)	37
A female GA mouse developing internal tumours causing 10% weight loss and slight abdominal swelling, while feeding a litter	20
A GA mouse with overgrown teeth that need regular clipping	8
None of these	3

Table 4. Delegates' views on 'severe' severity in relation to the breeding and husbandry of GA mice. Delegates could vote for as many of the options as they wished

	Number of votes
Yesterday	8
On Friday, today is Monday	4
This morning, it is now 4pm	5
2 hours ago	7
1 hour ago	8
'Found dead is always severe, no matter when last observed	37

Table 5. Delegates' answers to the question: 'would an animal found dead be classified as severe if they appeared normal when last seen:'

It appeared that most delegates regarded an animal 'found dead' as experiencing severe severity, on the basis of Tables 3 and 4 and also a question that aimed to explore whether the time that had elapsed between the last check, and the animal being found, made a difference (Table 5).

Action points

– Discuss the examples in Tables 3 to 5 at your establishment (or make up some more), to explore

views on severity and consider how effectively the assessment process is working. Include staff with a range of roles, discussing the reasons for different views.
– Think about how an 'informed choice' will be made as to the level of suffering that may have been experienced by animals found dead, on the basis of the type of procedures conducted locally.
– Encourage colleagues not to be 'defeatist' about the potential to reduce mortality, as progress can be made by refining monitoring and reviewing records to identify indicators that can be used as humane endpoints.

Acknowledgements

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