

# Report of the 2016 RSPCA/UFAW Rodent and Rabbit Welfare Group meeting

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## Introduction

The RSPCA/UFAW Rodent (and now Rabbit) Welfare Group has held a one-day meeting every autumn for the last 23 years, so that its members can discuss current welfare research, exchange views on welfare issues and share experiences of the implementation of the 3Rs of replacement, reduction and refinement with respect to rodent and rabbit use. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents and rabbits ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

The 23rd meeting was held at the University of Edinburgh on 1 November 2016 and was attended by 70 delegates from universities and pharmaceutical companies; most located in Scotland but some English facilities were also represented. Presentation topics included refinements in blood sampling rodents, reducing suffering in projects involving irradiation, vision in rodents, refinement and reduction via mixed-strain mouse housing, sharing information about good

practice, pain assessment in rabbits and ways of achieving human behaviour change to improve animal welfare. The Home Office Animals in Science Regulation Unit also provided some concluding comments. This report summarises the meeting and ends with a list of action points for readers to raise at their own establishments.

## Refinements in blood sampling rodents at Dundee

*Alison McNeilly, Division of Molecular and Clinical Medicine, University of Dundee*

Rodents, in particular mice and rats, are often the animals of choice in scientific research. However, the basic physiology and behaviour of each species is often not taken into account when designing and conducting experiments, which may have major implications for the scientific outcome, as well as causing avoidable distress to the animals.

In the fields of diabetes and obesity research, a common way to assess the severity of the condition is to measure the circulating levels of glucose, insulin and/or steroid hormones such as corticosterone in blood or plasma. Samples are usually obtained from the tail or saphenous vein or if larger sample volumes are required indwelling catheters may be surgically implanted into the jugular vein or carotid artery. Although these are all common practice, very little emphasis is placed upon basic physiology and the overall state of the animal during this sampling period. For example, blood glucose and corticosterone levels are directly related to how stressed the animal has become. With this in mind, we have developed a blood sampling protocol with an emphasis on keeping the animal as comfortable as possible in the sampling environment prior to blood collection. The aim is to ensure that the blood sample is as close as possible to one taken from the animal in their natural 'home cage' environment which should improve both the welfare of the animal and the science.

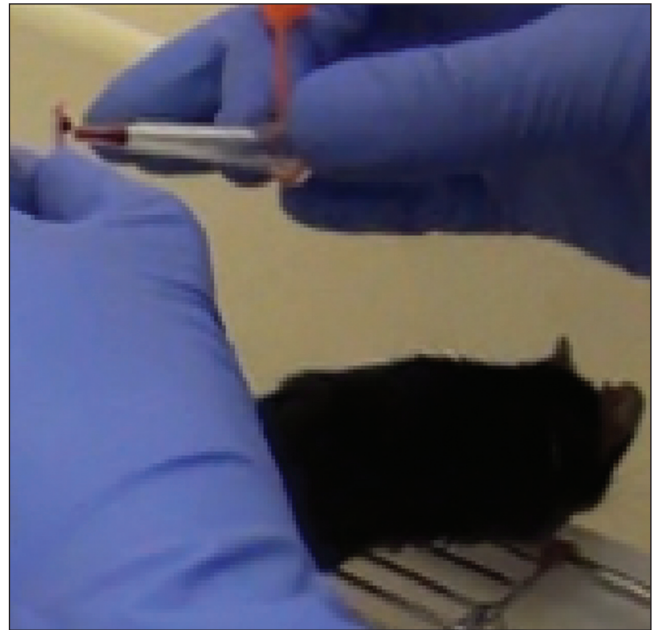
We try to ensure that the home cage environment is appropriate by housing in groups, providing environmental enrichment (e.g. a cardboard house, Nestlet™ and Sizzle Nest™), and we also capture mice by scooping them up or cupping them in our hands, as opposed to catching them by the tail.<sup>1</sup> Mice are habituated to daily handling, including time spent on a towel on a carer's lap (Figure 1). All of this helps to reduce anxiety and avoid the need for restraint when sampling.



**Figure 1.** Habituating mice to handling.  
Photo: University of Dundee

For small samples, e.g. for glucose assays, a needle (25 gauge) is used to prick the tail vein and produce a drop of blood which can be tested using a hand-held meter. We have found that blood glucose levels are significantly lower in mice sampled using this method. If larger samples are required, e.g. for hormone

assays, the mouse is allowed to move freely on a cage top and the tail vein is nicked using a scalpel. The tail is lightly held and 'milked' so that the blood can be collected using a capillary tube (Figure 2).



**Figure 2.** Blood collection from an unrestrained mouse.  
Photo: University of Dundee

These small changes in practice can have a profound impact upon the subsequent experimental read outs and the patience required to learn and begin using these techniques pays off in terms of reduced stress to both the animal and the sampler. We hope that our methods will also enable researchers to benefit from another 'Three Rs' – more Reproducible, Reliable and Robust data.

## **Bedside to bench – reducing the impact of irradiation on rodents**

*Jean Wilson, University of Glasgow*

When discussing the potential benefits of biomedical research, the phrase 'bench to bedside' is often used. However, it can also be helpful to 'turn the bed around' when using an animal model of a disease or treatment, by reviewing adverse effects in humans and considering whether and how these may apply to the animals. In this case, we examined how the side effects of radiation therapy in humans may be alleviated and whether this information can be of benefit to rodents used in research.

When humans and other animals undergo radiation therapy in the clinic, the irradiation is a treatment (either curative or palliative) and is usually targeted as opposed to whole-body. It is designed for the individual and there is a support team including a physicist,

dietician, physiotherapist and palliative care expert. In contrast, irradiation of mice as a regulated procedure is whole-body, performed to replace the bone marrow of one mouse with that of a different individual, is often done to study the effects of an alteration to bone marrow products and animals are treated in batches. We know that exposing mice to ionising irradiation causes breaks in DNA, affecting all cells and dividing cells in particular. This leads to radiation induced sickness or toxicity with systemic effects such as fever, hypotension, respiratory problems, shock, immunodeficiency, anaemia and gastrointestinal inflammation. Unless the bone marrow is reconstituted, the animal will take a long time to recover or would die without intervention. Bone marrow from another mouse, administered intravenously, is the usual source of replacement stem cells. Even with reconstitution, a fully functional immune response may not be evident for 30 days.

So, the overall protocol is much less sophisticated for mice and the support team is also reduced. What can we learn from patients at the ‘bedside’ to benefit the animals at the ‘bench’? We have found it helpful to consider clinical signs observed in human patients undergoing irradiation therapy alongside those seen in mice on preclinical studies (Table 1).

	Humans	Mice
Common clinical signs	Fatigue	Lethargy
	Nausea/vomiting	Gastrointestinal symptoms
	Inappetence	Weight loss
	Hair loss	Greying of the coat
	Long-term effects	Loss of body condition; delayed effects
	Graft vs. host disease	Graft vs. host disease
Signs that are not shared	Skin reactions	Cataracts
	Flu-like symptoms	Dehydration
	Fever Pallor/anaemia	Tooth loss
	Target-related symptoms	Swollen muzzle/head
		Death

**Table 1.** Clinical signs observed in humans and mice following irradiation.

Some of the clinical signs are common to both humans and mice whereas others are observed only in mice. Weight loss is probably the simplest side effect to identify and monitor but is also unfortunately the most difficult to control. Other key welfare indicators that we use are nest building behaviour,<sup>2</sup> the Mouse Grimace Scale<sup>3</sup> and body condition scoring.

Aside from minimising the adverse effects, as this is a potentially severe procedure, it is desirable to reduce variation in responses to the whole process, both to minimise wastage and to try to ensure predictable responses so that animals can be better supported. We employ a number of measures to reduce variation, including aseptic technique during stem cell collection, care of the cells and standardisation of the irradiation process. We have found that males are more robust than females and we monitor weight and body condition closely before and after irradiation. The irradiation source is regularly calibrated and we have Standard Operating Procedures in place that include sanitisation of the equipment and ensuring adequate training for those who use it. For reconstitution, asepsis is essential and we treat the process as a surgical procedure in that respect. During the recovery period, mice are housed in barrier caging with sterile consumables and easy access to soft food and acidified water. Extra nesting material is provided and body weight, condition and clinical signs closely monitored.

This is still something of a journey of discovery and we do not yet have all the answers but the question of whether we can improve the welfare of rodents undergoing whole body irradiation is still worthy of consideration and, if the experiences of humans can help – then all the better.

## Culture of share

*Andrew Brown, University of Aberdeen*

In the past, many animal facilities were quite insular, isolated areas, spoken of with a hushed sense of mystery and having little or no contact with the outside world. Unfortunately, there was also often a lack of communication *between* research and testing establishments with respect to sharing good practice about animal welfare and refinement.

We decided to tackle some of these issues and increase our outreach to other facilities, so that we can all benefit from better welfare and better science. We began with the premise that ‘sharing starts at home’, so we reviewed our own internal communications. Some issues were identified with between-staff communication and we felt that effective teamwork was sometimes lacking – generally because of a lack of time to focus on these. Our solutions included making time for staff talks (firstly by unit managers); joint social activities, regular meetings with feedback and rotating staff between units. All of these helped to develop and maintain closer cohesion and better communication between University staff.

The next step was to initiate a Scottish Technician Training Day in 2015, which focussed on training and assessments and included participation by the Home



Office. We are convening another meeting in 2016, which will cover training for Named Training and Competence Officers, handling assessments and environmental enrichment. Our Named Veterinary Surgeon (NVS) also initiated a Named Animal Care and Welfare Officer (NACWO) meeting, which was jointly held by the Universities of Aberdeen, Dundee and St Andrews and provided an opportunity to share and discuss common problems identified by NACWOs and solutions to these.

Moving on to include a wider audience, we have organised events and meetings with bodies such as the IAT, organised visits to (and exchanges with) other facilities and contributed to online discussion groups such as the Animal Welfare and Management Discussion Group (AWMDG) and Vets Online Email (VOLE), run by the Laboratory Animals Veterinary Association (LAVA). The University also signed up to the Concordat on Openness on Animal Research in 2015. This requires its signatories to be open about the benefits, harms and limitations of animal research and we have undertaken a range of activities and debates with local schools with positive feedback.

Our future plans include convening more meetings with other facilities to focus on refining specific areas (e.g. good surgical practice), further staff swaps between local units, more public engagement and possibly a new Scottish IAT branch. The Animal Welfare and Ethical Review Body (AWERB) hub system, recently set up by the Animals in Science Committee, could help to establish new contacts and collaborations. We would strongly encourage readers to get together and communicate with other facilities as much as possible and not to be afraid of public engagement.

## **The potential usefulness of thermography for assessing post-operative pain in rabbits**

*Caroline Krall, The University of Edinburgh*

A prominent challenge in rabbit welfare is the successful identification and alleviation of pain. Pain is defined as ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage’.<sup>4</sup> It is sometimes suggested that ‘prey’ species, such as rabbits, may be adapted to not exhibit pain overtly, as this would draw the attention of predators – but this also makes it harder for animal technologists to recognise signs of pain. Alternatively, others take the view that so-called ‘prey’ species do show signs of pain but human observers are poor at recognising these.

Whichever approach to thinking about the issue is true, the outcome for the rabbit is the same; a risk that pain

will not be effectively detected and alleviated. This has given rise to research aiming to improve the ability to assess pain in the rabbit, including enhanced observation of whole body pain behaviours such as belly-pressing<sup>5</sup> and facial expression analysis using the ‘Rabbit Grimace Scale’.<sup>6</sup> However, mild degrees of pain may be more difficult to distinguish with these methods.

Infrared thermography has been increasingly used in both clinical practice and research as a novel method for localising pain.<sup>7-9</sup> It relies upon the fundamental principle that heat dissipates as infrared radiation, which can be detected with an infrared camera. A painful stimulus will cause increased heat by two mechanisms: first, by evoking an increase in blood flow and inflammation at the site of injury; and second, through activation of the sympathetic ‘fight or flight’ response, which leads to peripheral vasoconstriction and an increase in core body temperature. Thus thermography has the potential to capture both the local sensory effects of pain and the sympathetic-mediated emotional aspect.<sup>10,11</sup>

In this study funded by UFAW, we aimed to determine the usefulness of thermography as a measure of post-castration pain in 16 young male New Zealand White rabbits. The castration procedure was not done specifically for this study. It is a routine procedure to prevent aggression in group housed males and to facilitate rehoming. All of the rabbits were successfully rehomed following the project.

The anatomical locations of interest were the scrotum (as the site of injury) and the nose and ears (as these represent the rabbit’s primary means of thermoregulation). Thus, these are likely candidates for detecting subtle changes in core body temperature.<sup>12</sup> Furthermore, a rodent study has demonstrated an increase in facial surface temperature but decrease in the temperature of peripheral locations (e.g. ears and paws) following foot shock using thermography. It was suggested that this change may represent a correlation with the affective (emotional) state of pain, as a central increase but peripheral decrease in temperature reflects an hypothalamic-pituitary-adrenal axis mediated vasoconstriction during a stress response.<sup>7</sup> Therefore, we hypothesised that rabbits treated with a ‘standard’ analgesia regime would exhibit higher facial and scrotal temperatures, as a reflection of increased pain post-operatively, than a novel multimodal regime which was thought to be more effective.

Subjects were randomly allocated to one of two analgesia groups: standard (0.2 mg/kg meloxicam s/q) or multimodal (0.6 mg/kg meloxicam s/c, 0.02 mg/kg buprenorphine s/q, 5% lidocaine/bupivacaine local infiltration). The study was divided into four phases: Baseline, Anaesthesia Only (so to differentiate

physiological changes due to anaesthesia versus surgery), Surgery, and 24hrs Post Surgery. Using a FLIR® One infrared camera with iPod® Mini, images were captured for each phase in the morning and afternoon and analysed using FLIR® Tools v. 3.7.0, focussing upon the ears, nose and incision site.

This research will be published in full later this year, so only the outcome will be summarised here. The results suggested that the standard analgesia group may have experienced mildly more pain than the multimodal analgesia group, as was evident by significantly lower apex temperatures. This may reflect increased pain as we would expect pain to induce vasoconstriction (thus lower temperature) in peripheral locations such as the ear apex. In addition, temperatures decreased significantly 2hrs post-operatively in all facial landmarks irrespective of treatment which suggests thermography captured a well-known consequence of surgery – hypothermia – and serves to verify the accuracy of thermography.

In conclusion, thermography shows the potential to be an easy method for detecting subtle physiological alterations that may be related to pain but further validation (e.g. alongside behaviours and facial expressions) is required to determine whether the changes in surface temperature observed were indeed reflective of pain and not another underlying physiological difference or emotional state.

## Caring and Sharing

*Jennifer Rees, on behalf of the CRO Rabbit Working Group*

Rabbits are widely used in biomedical research and in the safety assessment of potential new drugs and it is helpful to share good practice for refining rabbit care and use between facilities. However, contract research organisations (CROs) and sponsors sometimes feel restricted with respect to information sharing due to the highly competitive and proprietary nature of drug development.

In response to this, veterinarians and animal technologists within the CRO and pharmaceutical industries convened a working group to challenge misconceptions regarding what could be shared and communicate ideas and good practices to benefit rabbit welfare within the constraints of Good Laboratory Practice (GLP) and non-GLP studies.

Setting up a working group to share good practice may seem simple but there were high level management permissions to be sought, processes to go through to enable personnel to visit one another's facilities and limits to define regarding what could, or could not, be

shared. However, all of these issues were successfully overcome, despite the organisations being both customers and competitors, with senior management embracing and actively encouraging the process.

So far, we have set up a collaboration between several CROs that now work together to improve primate welfare and the success of this initiative gave rise to the CRO Rabbit Working Group, established in 2016. We aim to have monthly discussion groups which meet by teleconference with at least one representative from each company on the line. Minutes are kept and circulated to all. One CRO has also held a webinar on social housing of antisera rabbits, which was very well received. The Group has so far focused on regulatory assessment studies using rabbits, including general housing and husbandry (including diet and bedding), social contact and group housing, environmental enrichment, dealing with inappetence, refining restraint and dosing and sampling techniques. Some examples of good practice that we have shared are set out below.

- **Social contact and group housing:** Many safety assessment studies require that animals are singly housed, e.g. if individual food consumption data are required or there is a risk of cross contamination between dosing groups. The welfare impact of singly housing social rabbits can be reduced by housing in large, circular, Perspex floor pens. As there are no corners, territoriality is reduced and it is impossible for dominant individuals to 'corner' another rabbit and the startle response is reduced because the rabbits can see animals in other pens and approaching animal technologists.
- **Environmental enrichment:** All the CROs provide enrichment, such as cardboard tubes, paper, nesting materials, 'toys' and food items including fresh greens. It may seem simple to give rabbits some kale or broccoli but in a CRO/GLP environment there may be considerations for biosecurity and study integrity that can complicate matters. Sharing approaches to providing enrichment that will not affect study integrity has helped us to speed up implementation. We have also shared protocols for evaluating enrichment, floor area and flooring substrate from a rabbit welfare aspect, using behavioural analysis and preference tests.
- **Refining restraint:** In the CRO industry, most studies involve administering a set quantity of material to an animal, which usually involves restraint. For many administration procedures, manual restraint by a caring, competent animal technologist is preferred by most of the CROs in the Group. If a restraint device is necessary (e.g. for infusions that take time to deliver), members prefer fabric 'wrapping' type devices. These are commercially available or some CROs have modified old inhalation restraint tubes by cutting out holes for

the rabbits' ears. Successful designs, which have enabled sampling and dosing while keeping the rabbits calm and preventing injuries due to struggling, have been shared among the Group.

The Rabbit Working Group venture has helped all of the members, as we now have a network of people to call on for help, support and exchange of ideas with respect to improving rabbit welfare in our particular working environment. We hope to continue to work together, sharing and caring and the Group would welcome new members from CROs and other types of facility using rabbits.

## Educating rabbit owners – how to change human behaviour

*Suzanne Rogers, Learning About Animals*

Rabbits are the third most popular pet in the UK with an estimated 1.5 million rabbits kept nationwide. However, they are often housed in ways that do not meet their physical or behavioural needs. For example, the 2016 Animal Wellbeing report published by the PDSA found that, although rabbits are a social species, an estimated 52% of rabbits are kept alone.<sup>13</sup> Another important finding relating to rabbit health was that 24% of rabbits are not fed enough hay/fibre. While there are clear and significant animal welfare issues associated with the keeping of companion rabbits, simply educating people about rabbit behaviour and welfare needs is sadly not enough to achieve the widespread changes in human behaviour that are necessary to improve the lot of the pet rabbit.

My organisation, *Learning about Animals*, aims to find constructive ways to change human behaviour to improve animal welfare in a wide range of human-animal interactions worldwide (see [learningaboutanimals.co.uk](http://learningaboutanimals.co.uk)). In 2010 to 2012, we explored ways of improving the welfare of pet rabbits by running three workshops on rabbit behaviour. These were attended by a mixture of rabbit owners, animal rescue staff and veterinary nurses. Although the numbers of attendees varied between events, the number of people who actually owned rabbits at that time was 20 for each event. This pilot study compared human behaviour change as a result of each of the three events which used different approaches to changing the behaviour of rabbit owners and carers.

**Group 1:** Received a day lecture covering rabbits' needs, enrichment, behaviour and training/handling. The event was a little interactive with questions asked of the audience but this element was minimal. **Group 2:** These participants received the same lecture as group 1 but this also included a strong interactive element

whereby people considered how their own rabbits' needs were being met in a personal exercise involving defining and drawing the 'cobweb of needs'. **Group 3:** These received the same lecture and activity as group 2 but with additional follow-up after the lecture. A 'newsletter' of updates was circulated to this group every month for six months after the workshop. The newsletter included further information about rabbit behaviour and shared group members' photos and experiences in implementing the changes they identified as being needed.

All attendees filled out pre-event questionnaires and also a questionnaire six months after their workshop about the way they kept their own rabbits. The questions covered housing, diet, whether the rabbits were kept in groups or singly and behaviour of the rabbit. The number of people who changed their behaviour regarding providing their rabbits with improved social and dietary needs was 25% in group 1, 35% in group 2 and 40% in group 3.

Event 3, the most interactive workshop with follow-up, resulted in the greatest behaviour change among rabbit owners. Although a more thorough study needs to be undertaken in this context, this pilot study suggests that, as with other sectors, educational events should be as interactive as possible and provide follow-up in order to change the behaviour of attendees rather than just raise awareness. This is relevant not just for pet owners but for any educational intervention where behaviour change is the aim. It would be interesting to see whether some of these principles could be applied to training with respect to the behaviour and needs of laboratory rabbits, including training for researchers and I will be exploring this further with animal technologists in a workshop at the 2017 IAT Congress.

## What do mice see? visual and non-visual effects of light

*Stuart N. Peirson, University of Oxford*

Light exerts widespread effects on the physiology and behaviour of all commonly used laboratory animals. As well as making vision possible, light also plays a critical role in many non-image forming responses, including the regulation of circadian rhythms and acute responses such as regulating sleep induction, pupil constriction, heart rate, hormone release and learning and memory.

In mammals, these responses are all mediated via retinal photoreceptors, including the classical rods and cones involved in vision as well as the recently identified melanopsin-expressing photoreceptive

retinal ganglion cells (pRGCs) that are involved in non-image forming responses to light.<sup>14</sup> Understanding the full range of effects of light on the laboratory mouse therefore depends upon an appreciation of the physiology of these retinal photoreceptors, including their differing sensitivities to both light levels and to different wavelengths (i.e. colours).

Mouse visual acuity is extremely poor; a mouse would actually be legally blind by human standards with the equivalent of 20:2,000 vision.<sup>15</sup> Mice also have different sensitivity to colour in comparison with human vision. Although it can certainly be argued that the sense of sight is significantly reduced in mice as opposed to humans, vision is still important and relevant to these animals and in many ways mouse vision is actually comparable to human peripheral vision.<sup>16</sup> Therefore, the effects on physiology, behaviour and welfare (and therefore on the science) of laboratory lighting regimes deserves serious consideration. Despite this, most guidelines for lighting in animal facilities are based upon the requirements of the staff rather than the animals.

Mice are nocturnal, so it would make more sense from both scientific validity and animal welfare aspects to study them when they are active during the night. One obvious solution would be to reverse the animal facility lighting system, allowing them to rest during a light phase overnight and to conduct procedures in their active dark phase coinciding with the human working day.

To this end, some facilities use red light during the day, according with the common belief that mice cannot see in red light but this is actually a misinterpretation of the data. Humans have red and green cones with peak sensitivities of around 565nm and 535nm respectively. By contrast, mice lack a red cone and have a green cone with a sensitivity of around 508nm (see reference 14). As such, mice are around twelve times less sensitive to red light than humans but the level of red light has to be very dim before mice are unable to see. This is often not practical for researchers or animal technologists to work under. This means that facilities employing 'reversed lighting' systems, with full spectrum lighting at night and red light (typically ~600 nm wavelength) during the day, are actually using cycles of bright and dim light from the mouse's point of view, not light and darkness. It has been suggested that sodium lamps (~589 nm) could be used to light facilities during the dark phase.<sup>17</sup> However, mice are still sensitive to such conditions with just one seventh of the sensitivity of the human eye. Whilst mice will reverse their activity rhythms under light/dim-light cycles so that they are more active under the dim-light condition, studies have shown that dim light at night can lead to a range of adverse effects, including altered metabolism,

immune function, cortisol levels and affective behaviour (or mood).<sup>18-20</sup>

In the absence of any better solutions for refining light regimes, it may be better to increase awareness of circadian changes and how these might affect animal welfare and scientific validity. For example, there are circadian effects on:

- **Learning and memory:** Different results (both improved and reduced) have been noted in a range of behavioural tests according to whether these are conducted during the light or dark phases or even under constant conditions.<sup>21-23</sup> Surprisingly, many studies in rodents actually suggest that performance is better during the inactive, light phase.
- **Visual function:** The retina also contains a circadian 'clock' which fine tunes vision to the requirements of day or night. Retinal cone function is reduced during the subjective night (i.e. the dark phase for mice), so behaviour and physiology that are dependent on cone-mediated vision will be affected.<sup>24</sup>
- **Sleep and arousal:** Nocturnal exposure to light will induce sleep but will also elevate the stress hormone corticosterone in a similar way to a physical stressor. Studies to explore how light can increase both sleep *and* arousal have found that blue (470 nm wavelength) light is highly aversive to mice, increasing arousal and corticosterone levels whereas green and violet light promote sleep. These responses are dependent upon melanopsin.<sup>25</sup>

To conclude, it is still not clear how to achieve a 'naturalistic' lighting regime in the laboratory that is compatible with both human and mouse physiology and requirements. Whilst reversing light/dark cycles seems an obvious solution, this requires some form of lighting during the dark phase for staff to work under, which unless very dim, may exert effects on physiology and behaviour. Moreover, testing mice during their inactive phase is not always optimal. Recognition performance in behavioural tests seems to be better during the inactive (light) phase, whereas retinal function is impaired during the active (dark) phase and exposing nocturnal animals to light during their dark phase may act as a stressor, depending upon wavelength (colour). One statement that can be made with certainty is that it is essential to time-stamp experiments and state the lighting regime when reporting animal use, so that others can interpret the results, avoiding the need to repeat animal experiments.



## **Refining animal husbandry and reducing animal numbers through mixed-strain housing of laboratory mice**

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All common identification methods for laboratory mice (e.g. ear notching) can impair animal welfare. Furthermore, many experiments use genetically homogenous populations which inadvertently contribute to reduced external validity and poor reproducibility. We conducted a study that aimed to validate mixed-strain housing as a way to not only remove the need for marking but also increase variation in the study population and use a more statistically powerful experimental design (because every strain is represented in every cage, the number of independent replicates is increased).

We raised 3 to 4 week old female mice of three inbred strains, C57BL/6, DBA/2, and BALB/c, in single-strain or mixed-strain trios and in two housing treatments, standard and enriched. At 3 to 5 months of age, mice were assessed for 26 different behavioural (e.g. stereotypies), physiological (e.g. blood glucose) and haematological (e.g. white blood cell counts) variables. A diverse set of variables was chosen to make the results as applicable as possible across disciplines. Variables were analysed using general linear models that included: cage as a random effect, strain, cage type (single or mixed), enrichment (yes or no) and all of the interaction terms.

Single- and mixed-strain housed mice did not differ in any measured variables. Several strain differences were detected and all of these were as expected. Furthermore, the magnitude and direction of typical strain differences was unaffected by whether or not animals were housed with same-strain cage mates (there were no interaction effects). Enriched mice showed signs of improved welfare (e.g. less stereotypic behaviour) and these effects were similar for both single- and mixed-strain housed mice. Thus, mice in mixed-strain trios retained their strain-typical traits. Mixed-strain housing also reduced inter-individual variation across all variables.

Ultimately, we were able to demonstrate that mixed-strain housing is a potentially valid experimental

paradigm with the following benefits: it does not involve any invasive or stressful procedures; it systematically increases variation in the study population which increases the generalisability of the results; and it increases the statistical power of the experiment by reducing inter-individual variation and increasing the number of independent replicates which means fewer animals need to be used in order to detect effects (in most cases we estimate less than half).

## **Closing comments from the Home Office**

*Kathy Ryder, Animals in Science Regulation Unit (ASRU)*

Many people strive to improve the welfare of laboratory animals, either using entirely novel means or by small improvements to existing methodologies (the so-called 'marginal gains' approach). Good work in this area is often presented at meetings such as this one. However, what happens after the meeting? Decisions need to be made as to which suggestions are relevant, feasible and worth trialling or taking up. It is also important to have protocols in place for evaluating potential refinements from the aspects of animal welfare, impact on the science and resource implications. Any downsides, such as increased resource requirements or even increased animal numbers associated with reduced severity, will need to be considered carefully against the benefits.

In the real world, accountants play a role in determining how much resource is made available for implementing refinements to procedures, housing, husbandry and care with the key question '*is it worth it?*' likely to arise. It is therefore essential to ensure that animal welfare and the scientific benefits that accrue from better welfare, are given due consideration.

Animal technologists can play a role by helping to build the case for better welfare leading to better science and by disseminating knowledge about refinements and their benefits as widely as possible.

The role of the Named Information Officer may be key and other relevant people, such as the Named Training and Competency Officer, may be very valuable to progress acceptance of new or revised methods. Availability of training on new technologies can also help teams to achieve take-up of new, improved techniques and approaches. This need not always mean producing a formal publication; see Table 2 overleaf for other suggestions.

Your local ASRU Inspector can also advise on refinements and how to evaluate, implement and



Method	Outputs	Advantages and disadvantages
Give a talk	Audience knowledge and motivation Meeting abstract	Great engagement and opportunity to discuss People forget! Abstracts are not always searchable
Present a poster	Audience knowledge and motivation Meeting abstract Flyer, if you produce one	Opportunity to engage and discuss if people come to view People forget (unless they have a flyer) Abstracts are not always searchable
Write a paper	Journal article	Greater potential to share, especially if open access Hard work Not always enough original science to publish
Discuss at the AWERB	Thought stimulated amongst members and their colleagues  AWERB minutes Potential feedback to AWERB Hub network	Opportunity to advise Establishment Licence Holder and change local practice  Audience may be local people who read minutes only – unless these are posted onto website
Talk to scientists	(Possible) adoption of the refinement Could stimulate wider thought about welfare and ethics of animal use	Focussed discussion Likely to affect one, or few, people only Reasoning for change not always clear without literature to make the case
Include validated refinements in training courses	Trained and motivated researchers, animal technologists and named persons	Long-lasting change locally Need to prepare materials and gather evidence

**Table 2.** Ways to communicate about refinement to different audiences.

disseminate these effectively. By working together, we can obtain a realistic picture of the relative value of improvements to animal welfare and monitoring and we can help create a web of information streams and contacts to help share good practice.

### List of action points based on all of the presentations and discussions

- Consider ways to reduce the stress associated with blood sampling, especially relating to restraint. Discuss the potential to use the method developed at the University of Dundee at your own establishment.
- If you are involved in studies including animal models of human diseases or treatments, consider how the adverse effects seen in humans and how these are ameliorated, could help to identify potential refinements.
- If projects that involve irradiating rodents are undertaken at your facility, see whether any of the approaches to refinement outlined in this report could be applied.
- Reflect on how effectively refinements are communicated both within and outside your facility, including raising this within the AWERB.
- Reach out to other establishments with respect to sharing good practice and respond if other facilities or AWERBs contact yours (including supporting the Animals in Science Committee’s AWERB Hub network).
- Use the section on thermography to assess post-operative pain to start a discussion on welfare- and pain-assessment at your establishment. Is there an effective mechanism for keeping up with new techniques, approaches and applications?
- Explore ways of sharing validated good practice, including contacting the CRO Rabbit Working Group if relevant.
- Find out more about behaviour change theory, e.g. via Learning About Animals and think about the potential to apply this to in-house training and interactions with other staff.
- Ensure that your establishment keeps up with the literature and thinking about the effects of light on laboratory rodents and rabbits, especially nocturnal animals. You may like to raise this as a topic for the AWERB to discuss, including the importance of reporting lighting protocols and the timing of experiments.
- Be aware that rodents can see in red light, so do not perceive red light as ‘darkness’ or red tinted nest boxes as opaque.
- Be aware also that many ‘reverse light-dark regimes’ are actually ‘bright and dim light regimes’ with consequences for animal physiology and welfare.
- Discuss the potential to use a mixed-strain housing protocol at your establishment.

- Commit to personally disseminating evaluated welfare improvements in a range of ways, including working with your local Home Office Inspector.

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