Report of the 2012 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.

The 2012 meeting addressed a range of topics including rodent use in Chile, caring for aged mice, reducing stress during blood sampling, the welfare impact of different identification methods and implementing the Three Rs in antibody generation. The meeting also discussed refinement and translatability issues with respect to selecting the appropriate age of rodent for *in vivo* studies and to rodent studies of neuropathic pain. The day ended with a focus on refining severe (substantial) procedures, with talks on an RSPCA initiative to reduce severe suffering and a practical example of refinements for SOD-1 mice, a genetically altered strain used to study neurodegenerative disease.

Experimental animal use in Chile

Jess Gimpel, Pontificia Universidad Católica de Chile

Chile has a population of 17 million people and over 60 universities. Nearly a third of these have medical faculties, half have schools of veterinary medicine and there are a similar proportion of biology, pharmacology and biochemistry schools. Most of these entities do at least part of their research using animals and some of them base most of their scientific output on animal experiments. There are also 'bio-companies' and non-profit research organisations that use animal models. About 15 registered rodent vivaria exist but it is still common practice for some researchers to have a small, private animal colony. Currently, there is only one vivarium in Chile accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). It achieved accreditation this year and there is no other animal facility applying for certification at the time of writing, even though there are at least three major projects in the country that are setting up 'state of the art' animal facilities.

Until recently, Chile had one regulation relating to animal cruelty in its Penal Code that also had to be applied to scientific procedures using animals. However, this was a generic law and so was not a very satisfactory way of regulating research using laboratory

animals. An Animal Protection Law was sent to Parliament but then sat there for over 10 years. While the law was discussed, scientists and animal care professionals sought a way to implement voluntary standards specific to experimental animals in order to make progress in this area. In 2004, a Standard based on and modified from, ISO 10993-2:2006 (Biological evaluation of medical devices – Part 2: Animal Welfare Requirements) was proposed, the Chilean Norm NCh 2856/2. It was regarded as an important step at the time, however, there are still no vivaria currently certified according to that norm in the country.

In 2009, the Chilean Animal Protection Law (20.380) was finally promulgated. This regulates all animal use, including research. It defines what animal experiments are, where these may be performed and who can conduct them. An important step is that the law forbids the use of animals for school demonstrations. The law also required that a National Bioethics Committee, which would draft all the regulations, be established 60 days after its publication. Three years later, this committee is still not constituted; hence, the law, albeit now official, is still not ready to be properly implemented with regard to animal procedures.

Fortunately, things have moved forward despite the lack of a proper legal backup. This is mainly due to scientists training overseas, international collaborations and journal editorial requirements of bioethics approval before research can be published. A major incentive has also been provided by Conicyt, the main national research funding agency (www.conicyt.cl/), which introduced a requirement for an ethical review process to apply to all projects that include animal use. This year Conicyt has also started to apply procedures for retrospective reviews and project audits. Therefore, institutions have started to set up their committees and review procedures. In addition, more vivaria have started to hire veterinarians and animal technologists and professionals working in animal facilities have formed a national association, ASOCHICAL, which meets monthly and organises courses with national and international speakers (www.asochical.cl). These have been very successful and demonstrate both the needs and interests of those involved in experimental animal care and use to learn and to do things right. The future looks promising, although we still have a long way to go. There is still a great need for training, both at veterinary and technical level, as well as more awareness of scientists that good animal care is essential not only for the animals but also for their research to be sound.

Action points:

- Continue to make efforts to increase awareness among researchers of the importance of raising standards of animal care.
- Collaborate in supporting good practice when implementing regulations controlling experimental animal care and use.
- Increase training opportunities for all those involved in animal research and testing, especially animal technologists, with particular emphasis on providing resources in Spanish.

Neuropathic pain: Refinement and enhancing the clinical relevance of rodent *in vivo* models

Andrew Rice, Imperial College London

Neuropathic pain is a type of chronic pain that is caused by a lesion or disease affecting the sensory nervous system[1]. Following the initial trauma or disease which damages the nervous system, neuropathic pain may occur in the absence of any noxious stimulus. Neuropathic pain has no apparent biological function, but is usually both severe and chronic – and with a population prevalence of about 7%, and few effective therapies, it presents an area of therapeutic need.

Although a number of novel treatment approaches have been developed in recent years, in general their analgesic efficacy can be limited, and their effectiveness varies between patients. As a result, there has been considerable effort to develop new drugs to alleviate neuropathic pain. These efforts include animal use in studies of peripheral nerve injury which have helped to identify some putative pain-generating mechanisms as potential drug targets. However, in terms of *predictive validity*[2], these animal studies have limitations with respect to 'forward translation' (predicting the efficacy of new agents in clinical trials), because they are not good at screening out compounds that go on to fail due to lack of efficacy. In fact, nearly all the drugs currently in use to alleviate neuropathic pain were initially developed for other conditions or discovered by chance.

There are also a number of ways in which the standards of experimental design and reporting of animal studies of neuropathic pain can be improved (see references 1 and 2). One critical generic issue, which pain shares with other therapeutic areas, is the need to refine experimental design to minimise the impact of experimental bias in overestimation of treatment efficacy (e.g. concealed allocation, sample size calculation, observer and analyser blinding, reporting of withdrawals and drop outs and clear stating of pre-determined inclusion and exclusion criteria etc.)[1-4]. Furthermore, animal studies should be reported in a format which includes all relevant information and allows the reader to easily ascertain

[1] This produces perceptions of phenomena such as touch, temperature, body position (proprioception) and pain.

[2] The ability of a model to correlate with, or predict, a future performance or variable.

the methodological rigour with which an experiment was conducted[1,2,5].

It is obviously critically important to use the most appropriate study designs and protocols for *in vivo* research, otherwise animals will be wasted – which is a serious ethical issue. There are a number of new approaches to neuropathic pain studies that aim to both reduce animal suffering and improve translatability. For example, neuropathic pain in humans is not only caused by physical trauma, but can be associated with a variety of different pathologies. Traditionally, animal models of neuropathic pain are of peripheral nerve trauma, whereas human clinical trials tend to be conducted in painful polyneuropathy (e.g. diabetic neuropathy) or post herpetic neuralgia (chronic pain complicating shingles). In order to correct this dichotomy between the animal and human 'clinical trials', an increasing number of animal models of neuropathic pain are now emerging which better reflect the range of clinical conditions that can provoke neuropathic pain.

Another relevant consideration is the type of clinical signs that are used in animal studies to assess neuropathic pain and the efficacy of any potential therapies. Of course, an emotional experience like pain can be self-reported in humans but can never be directly measured in rodents; we can only infer the presence or absence of pain from other, usually behavioural, observations. 'Traditional' outcome measures such as limb withdrawal to sensory stimuli (e.g. a mechanical stimulus, heat or cold) are not especially translatable to the human condition, in which there are other co-morbidities such as anxiety, emotional disturbance and interference with the normal circadian rhythm. However, natural behaviours that are ethologically important in the world of the rat can provide more useful outcome measures. For example, rodents in pain may display behaviours which decrease their exposure to predation risk; for example in open field tests they may perform more 'risk assessment' behaviours (such as rearing) and spend less time in the centre and more time in contact with the walls (thigmotaxis). These behaviours have also been used to pre-clinically evaluate potential anxiolytic drugs. Other behaviours such as spontaneous burrowing (a sort of 'house-keeping' behaviour), in which rodents dig into gravel- or food-pellet filled tubes in the home cage (Figure 1), is also reduced in association with a number of pathologies including neuropathic pain[6].

Using 'outcome measures' that are more relevant to the animal can not only help to improve translatability, but can also provide more sensitive indicators of suffering; for example, behaviours such as burrowing may be altered before there are any clinical signs of suffering that are apparent to the human observer. This approach could thus also be used to guide refinements such as implementing earlier humane endpoints.

Figure 1. Mouse in a burrowing tube
Photo credit: Image from Deacon, R. Assessing Burrowing, Nest Construction, and Hoarding in Mice. J. Vis. Exp. (59), e2607, DOI : 10.3791/2607 (2012).

Action points:

- For **researchers**: review the kind of outcome measures used to assess pain within pain studies, especially neuropathic pain. Would there be scope to change to more of a 'natural behaviour' approach?
- Consider whether the approach to creating the animal 'model' of neuropathic pain could be refined.
- Use a rigorous 'Good Laboratory Practice' approach to experimental design and conduct, in an effort to minimise experimental bias. Report experiments which used animals according to the ARRIVE guidelines[5].
- For **animal technologists**; consider whether the more 'animal centred' outcome measures could be adapted to help assess suffering in other species and study types. You might like to bring the topic to your local Ethical Review Process (now the Animal Welfare and Ethical Review Body) for discussion.

Survey of the welfare impact of different identification methods

Dominic J Wells and Nur Mazlan,
Royal Veterinary College

A number of different methods exist for identifying mice, including noting natural markings, ear punching and notching, ear tags, microchips, tattooing, clipping or dyeing fur and toe clipping. These have varying degrees of invasiveness and toe clipping is the most controversial and is widely regarded as being of special

concern, due to the potential for both pain and loss of function. It is accepted that the method with the least possible impact on the animal should be used in each case, but there has been only limited investigation of the welfare consequences associated with different methods. Given the huge number of mice used in experimental procedures annually and the need for the majority of them to be unambiguously identified, the choice of identification method for mice can have a significant impact on animal welfare.

In the first half of 2012 we performed an on-line survey of current identification practices in animal facilities in the UK, receiving 60 responses out of a possible 83. Facilities that replied included academic institutions, government research institutes, pharmaceutical companies and contract research organisations. Respondents were mostly facility managers or senior animal technologists (Named Animal Care and Welfare Officers). Ear punching or notching was the most common permanent identification system and marker pens were the most common temporary system. A number of institutions had ceased to use certain methods including microchips, ear tags, tattoos and toe clip (Table 1). Mice were most commonly marked between 2 and 4 weeks of age (61%), with only 7% marked at under 2 weeks old. The main criteria for selecting a method were reliability, minimal animal welfare concern and ease of reading. The high use of ear punch/notch may reflect the current use of this method as the preferred technique for obtaining DNA samples for genotyping genetically altered mice, although tail biopsy is still commonly used. Toe clipping (i.e. amputation of the distal phalanx) was not favoured due to concerns about animal welfare and was used by under 5% of establishments, for example in cases where it was felt that the practice could be justified due to a risk of a lethal phenotype that resulted in the death of the animal in the first days of life.

Discontinued methods	Reasons given
Microchip	Cost, welfare concerns, loss of chips
Ear tag	Welfare concerns, not reliable, hard to read after some time, not easy to identify at a glance
Tattooing	Unnecessary, too 'fiddly', caused local inflammation, other less invasive methods are available
Toe clip	Unnecessary, welfare concerns, not easy to identify visually without handling
Marker pen	Only for short term studies, cannot be used on black mice
Ear punch	Difficult to do and read, changed to microchip (database linked), not easy to identify at a glance
Hair dyes	Not permanent, impractical, other reliable methods are available
Fur shave	Impractical
Bar coding	Not reliable

Table 1. Discontinued mouse identification methods, with reasons: Just over half the responding establishments reported making a decision to stop using one or more identification methods. These are listed in the table, with the most commonly discontinued method first (microchip, 25% of establishments that responded) and the least appearing last (bar coding, under 5%).

We are currently further investigating the welfare impact of the different methods of identification. It is necessary to consider each technique from the animal's perspective, as even apparently innocuous methods can be stressful. For example, temporary identification can be achieved by the use of hair dyes or indelible marker pens. This appears to be harmless, although the solvent does appear to stress rats[7]. Importantly, these and other procedures involve capture and restraint of the animal which is itself stressful – and may actually be the major stressor associated with many identification techniques[8]. However, it may be possible to reduce restraint stress by refining capture and handling, for example by catching in the home cage tunnel or in cupped hands[9], or by avoiding scruffing when using marker pens. Reviewing the whole process from the animal's point of view in this way is a useful approach to refining identification and one that we encourage.

Action points:

- All identification methods (apart from natural markings) will cause a degree of stress; so keep a watching brief of new ideas to refine them.
- In general, use the least invasive possible technique, but also take account of the duration of the project or breeding programme. Repeated application in the long term might actually cause more suffering than a more invasive, one-off identification procedure.
- Staff experience and expertise have major influences on the animal's experience, so ensure that training is adequate for those applying identification techniques.
- If genotyping is required, consider combining biopsy and identification procedures, e.g. using ear notch or punch material for genotyping.

Making the right choice of age for rodent *in vivo* studies

Judi Latcham, GlaxoSmithKline

The choice of animals used in efficacy studies is a

critical factor in determining data quality. Criteria such as choice of species, strain and sex are generally embedded in the decision making process. However, with some exceptions (e.g. drug efficacy studies for neurodegenerative diseases), the age of rodent used in efficacy studies is generally restricted to the early phase of their lifespan, e.g. when the animals are about six to eight weeks old or at a set weight.

The absence of a clear scientific rationale for using this age range raises concerns about both the optimisation of rodent models for efficacy and their relevance to the clinical setting. It also has ethical and economic implications, if animals past the desired age are wasted because they are no longer considered to be suitable. GSK has set up a project that aims to scientifically evaluate the effect of rodent age on data quality in efficacy studies and also aims to raise awareness about the choice of age in rodent models used in research.

Although most strains of mice are *sexually* mature at 35 days old, relatively rapid maturational growth continues, for most biological processes and structures, until animals are about three months old. This means that 'mature adulthood' in the mouse lies between three and six months of age. Mice are 'middle-aged' between 10 and 14 months, and considered 'old' between 18 and 24 months[3].

These life stages should be taken into account as part of good experimental design. For example, the immune system of the mouse does not reach maturity until adulthood. An immunological study of the T-dependent IgG response in CD1 mice found that the response was significantly less variable in 16 week old animals than in 7 week olds (I Holyer, personal communication.). Results such as these are of extreme importance and have had a significant effect when deciding which compounds to progress.

There are also implications for the Three Rs and the culture of care, for example:

- Greater awareness of the importance of study design.
- More critical scrutiny of statements such as "we have always done it this way ...".
- Reductions in animal numbers, for example due to reduced variability and better quality data.
- Enhanced clinical relevance, thus reducing wastage.

The outcome of the GSK project will be included in a comprehensive set of guidelines for the design of high quality animal studies. A group co-ordinated by the UK National Centre for the Three Rs (NC3Rs) will continue to collect data throughout 2013, to bring the project to a conclusion.

Action points:
- Raise awareness among colleagues of new thinking about mouse life stages.
- Consider and factor in the stage of maturity of mice during study design; ensure that the correct age is selected.
- Challenge study protocols that state they will use 'mature' mice aged 35 days.

[3] See http://research.jax.org/faculty/harrison/ger1vLifespan1.html, last viewed 10 January 2013.

Refining blood sampling in the rat

Kate Heath, GlaxoSmithKline

As part of the process of drug research and development, blood samples are sometimes needed from rats immediately post-dose. Historically, this was done via the caudal vein, after whole body warming for approximately ten minutes in a cabinet at 39˚C. The rats were then restrained during blood sampling to avoid injury and ensure an effective procedure, but the 'slanted table' restraint technique used at the time required two operators, was stressful to the animals and so was unpopular with staff. There was an obvious need to refine this procedure and so the decision was made to find an alternative approach.

We found that another establishment was using a refined method of restraint for less stressful blood sampling from the jugular vein, which led to a series of training visits in which we gained competence in the new technique. Correct animal handling is the key to success in this method and it took 24 hours training in total to learn to restrain the rat's forepaws and make a 'finger stool' to support the head.

Once animal handling and restraint were assessed as competent, we moved on to learning the sampling procedure. Training began with a 'butterfly' or winged needle with flexible line (Vygon UK, Swindon) which ensured that the hand was kept steady. A second person was required to do the sampling in the initial stages and we were then able to move on to performing the technique without an assistant, by restraining the rat with one hand and sampling with the other. We were finally assessed for competency in successful sampling on both sides, so that these could be alternated in the case of multiple sampling.

There was some scepticism about the feasibility of the new approach among staff when we brought this back to the facility, so we began by training two volunteer members of staff who were especially experienced in blood sampling, with a benchmark for competence of about 20 successful samples.

Once we started to implement the new technique at our

establishment, we reviewed its benefits over the method we had used previously. We found that the manual restraint and jugular sampling route had a number of benefits; for example, the rats were calmer during sampling and showed no signs of distress afterwards, the operator found it less stressful because the rats tolerated the procedure better and it was also faster to perform (about one minute as opposed to three to four minutes for table restraint). Stress to the rats was further reduced because there was no need for whole body warming or restraint apparatus.

We also made some changes of our own to the protocol. Shaving was discontinued, as it risked clipper abrasions without providing any health benefits. It was also found that nitrile gloves made it more difficult to handle large rats confidently so we changed to powder free, Aloe Vera latex gloves with a textured grip (low protein 50 µg/g, beaded cuff, 4-5 mm thickness). Sterile water was also used to part the fur instead of alcohol.

Using the new method, we can sample blood immediately after dosing, which provides scientific benefits as well as – most importantly – reducing stress to the animals[4]. We believe that opening our minds and embracing new ideas can really improve the welfare of laboratory animals and we have gone on to introduce this technique for other studies in Toxicology Support Safety Assessment.

Action points:

- Revisit routine protocols for procedures such as blood sampling and consider whether these could be further refined.
- Explore the potential to use expertise at other establishments when researching refinements and learning new techniques.
- Always review and evaluate refinements to ensure that animals are benefitting and staff are comfortable with new techniques and feel competent to conduct them.

[4] Note: All animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Caring for aged mice and assessing their welfare

Mark Gardiner, Mary Lyon Centre, MRC Harwell

People are living longer, so diseases associated with the ageing process are consequently placing increasing social and financial burdens on society. During the past three years a new project has been initiated at MRC Harwell which focuses on diseases related to ageing with the aim of improving quality of life in old age rather than increasing longevity. This project has two parts; (i) to comprehensively phenotype a selection of common inbred mouse strains at specific ages and (ii) to use large-scale chemical mutagenesis to generate lines of mutant mice. These mice are being screened throughout their lives for diseases that are prevalent in ageing populations such as diabetes, sensory deterioration, neurodegeneration and osteoporosis[10].

As our animals are living for much longer than the average laboratory mouse, we need to be able to care for them appropriately. This includes recognising the difference between a healthy, aged animal and one with welfare concerns or imminent health issues. We designed tailored welfare assessment protocols in consultation with senior animal technologists, researchers and the veterinarian, using the Mouse Welfare Terms database of clinical signs (www.mousewelfareterms.org).

In addition to monitoring the appearance and progression of age-related ailments, the welfare assessment system has also been used to chart the changing appearance of healthy ageing animals. We have collected profiles demonstrating how body condition, coat appearance and weight all change during the life of a mouse and how this varies between sexes and strains. This has allowed us to refine our welfare assessments and to ensure the best scientific outcome from these animals, whilst providing the most effective care for them.

Some of the phenomena observed as mice age include:

- Effects of **dominance behaviours** can become more marked as the animals age. Mice of social strains are housed in groups of five because of the welfare benefits associated with housing social animals together, but a degree of dominance is inevitable. Over time, this may be evidenced by different body weights between female cage-mates as they age. This need not necessarily be a welfare problem but animals are carefully monitored so that judgements can be made on a case by case basis.
- **Barbering**, in which fur or whiskers are pulled out by the 'barbered' animal or one or more other animals (usually in a repetitive pattern), may also occur more as animals get older. Groups are observed closely if signs of barbering occur and any sore patches are treated topically. Sometimes it is appropriate to remove the 'barber' and animals are humanely killed if their sores do not resolve.
- As opposed to barbering, **natural hair thinning** is commonly seen in older mice and we do not now see this as a welfare concern provided that the skin is unaffected (Figure 2). Like humans, aged mice can lose hair colour; for example black mice may go grey or ginger.

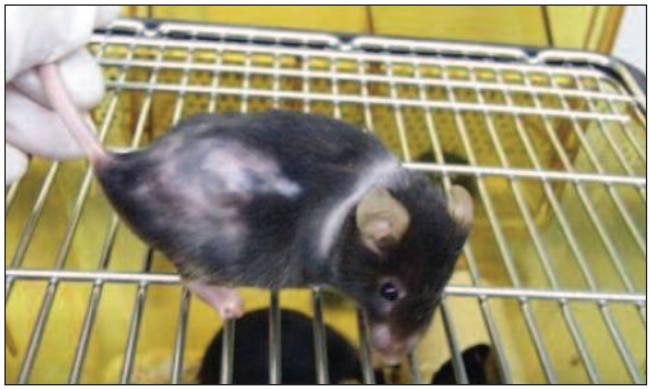

Figure 2. Thinning coat on a healthy, aged mouse
Photo credit: Mary Lyon Centre, MRC Harwell

- **Weight increases or decreases** may also be observed as animals get older. Larger mice are handled carefully, as for a heavily pregnant female and their weight is taken into account if there is a requirement to anaesthetise them. A general endpoint of 15% weight loss is in place for aged animals unless there is scientific justification for maintaining them, in which case a maximum weight loss of 20% may be deemed acceptable.
- **Eye abnormalities**, such as swelling, exophthalmia and very cloudy eyes are sometimes observed as mice age. These are generally very rapid in onset and affect one eye only and animals with these kinds of significant eye problem are humanely killed. Cataracts, in which the cornea becomes opaque and there is a blue tinge to the eye but no swelling, are monitored closely and animals euthanased if there are signs of distress or the condition progresses to become more severe as described above.
- As with many other species, aged mice are prone to **external growths** such as warts or abscesses. Abscesses are generally due to *Staphylococcus* infection, and if they do not appear to be painful or interfering with movement, may be treated by lancing, using a local anaesthetic cream to control discomfort or pain. There are no treatments for warts and other growths, so animals with these are closely monitored to see whether there are any signs of discomfort or interference with feeding, drinking, normal posture or movement, in which case the animal is humanely killed. Animals with abscesses are also euthanased if the above applies, or if the abscess does not clear up following treatment.
- **Malocclusion** of the teeth can become more prevalent in aged mice, or within the mutagenesis programme. If appropriate, this can be treated by trimming the teeth (using a new design of clipper we have found that is more effective and less stressful for the mice) and providing wet mash. If the animal begins to lose weight and there is no improvement, they are euthanased.

Animals previously housed in groups are not left alone if their numbers have been reduced due to barbering or other humane endpoints; they are used in terminal procedures or for tissues. More aggressive strains such as BALB/c are no longer used, because we find they generally have to be singly housed and this would be for long periods of time in ageing studies. It is very important that care is taken to review the animals' behaviour and needs throughout their lives, especially during the ageing process, and that enough time is allocated to the care of ageing mice – and this is built into research programmes.

Action points:

- Consider how long animals live in your establishment – are any of them 'aged' and if so are their needs being catered for?
- If experimental protocols for social animals of any age involve individuals being permanently removed, review whether provisions are in place to ensure that animals are not left alone.
- Look at the Mouse Welfare Terms database and consider using its approach to observing and describing mice.

Evolution of antibody generation at GSK

Trevor Wattam, GlaxoSmithKline

GlaxoSmithKline generates monoclonal and polyclonal antibodies as tools for research, reagents for clinical assays and as therapeutic reagents for the treatment of disease. The Antibody Generation Group (AGG) within GlaxoSmithKline has always focussed on the generation of high quality antibodies, with strong emphasis on creating and maintaining a good culture of care when using animals. Our goals are to use the minimum number of animals, with the minimum number of immunisation procedures per animal, causing minimum distress. We also aim to maximise use of animal tissues.

The general culture of care also includes making empirical observations on the outcome of projects and discussions of these between the AGG, relevant project teams and the Laboratory Animal Science Team, all of which communicate regularly. This has led to changes in immunisation strategies over successive project licences and refinement of the antibody generation process. The current process can be summarised in these three steps:

1 An *Antibody Generation Proposal*, in which a request for the generation of an antibody is presented to the AGG. This must include the type of antibody, its target specificity and end user applications. The proposal is discussed to confirm that the antibody

reagent is suitable for its application and that valid and valuable research data would be obtained. The AGG also checks for pre-existing reagents, both internally and externally, and no new antibodies are generated if pre-existing sources are found.

2 An *Expression Strategy Meeting*, including the requestor, the AGG and others with relevant expertise, e.g. in gene cloning or protein purification. This meeting discusses the target antigen type, immunisation strategy, screening methodology, reagents required for immunisation and screening and the time line to generate the antibody. Immunisations do not start until all reagents and screens are in place.

3 *The immunisation protocol* – GSK has a 'default' protocol with respect to immunisation sites, doses and timescale. This includes refinements such as low immunogen doses and careful selection of adjuvants, resulting in reduced adverse effects, better serum titres and fewer immunisations (Freund's complete and incomplete adjuvants are no longer used). Discontinuing the use of 1 inch 23 gauge needles and replacing these with 0.5 inch 26 gauge needles, has improved precision when immunising as well as reducing discomfort to the animal.

This process has led to 50% reductions in animal group sizes, from four to two animals for all immunisations. The refinements that have been implemented in immunogen preparation and immunogen delivery have allowed the AGG to maintain its success rate whilst performing all immunisation procedures under a mild protocol severity. Adverse events are very rare, occurring in less than 1% of immunisations. However, we believe that refinement never stops and aim to implement more improvements to the immunisation protocol that we hope will replace some animal use and further reduce the number of immunisations necessary. These include DNA immunisation methodologies, new adjuvants or combinations of adjuvants, targeted immunisation approaches and complementary *in vitro* antibody generation techniques.

Action points:

- Ensure that there is a process in place for continually applying the Three Rs to 'routine' procedures such as antibody generation.
- Make sure that all relevant staff and groups communicate with one another and share information on the Three Rs with respect to antibody generation with other establishments.

Refining severe procedures

Elliot Lilley, RSPCA Research Animals Department

Ending severe (substantial) suffering is a top priority for the RSPCA. The Research Animals Department has recently increased its efforts to develop and promote ways of avoiding or reducing severe suffering. We aim to identify:

- the kinds of procedures that can cause severe suffering;
- the factors that combine to make the level of suffering severe, such as pain, anxiety, or long lasting procedures;
- the purpose of severe procedures, for example some vaccine tests or studies of painful or distressing disorders;
- any perceived or actual obstacles to reducing suffering or avoiding these procedures, and most importantly;
- what can be done to overcome these?

We are currently visiting a wide range of establishments to discuss the project, collect case studies from those who have successfully avoided or reduced severe suffering and encourage people to pass on good practice in publications, through discussion with colleagues and at scientific meetings.

It is a particularly good time to tackle this issue as the need for better recognition and assessment of animal suffering – particularly cumulative suffering – is increasingly recognised and the new Animals (Scientific Procedures) Act will require assessment of the actual severity of procedures, with reporting in the annual Home Office statistics.

The RSPCA's work on severe suffering comprises three broad 'strands'. The first is identifying those procedures that have the potential to cause severe suffering and setting up a series of expert working groups (operating in a similar way to the Joint Working Groups on Refinement) to develop and promote refinements to these. This work also builds on previous projects looking at recognising pain, suffering and distress in laboratory animals and welfare assessment protocols.

We are aware that many potentially severe procedures have already been refined so that they cause less suffering, but much of this important work has not been effectively published or disseminated. With this in mind, the second strand of the project is to collect examples of severe procedures that have been refined, so as to (i) help disseminate these and encourage wider uptake of the methodologies used and (ii) see whether there are any common approaches that might be applied to refine other severe procedures.

Thirdly, we are encouraging local ethical review processes to consider a 'stretch objective':

Could our establishment achieve an end to severe suffering, either by refinement of existing approaches

or by avoiding the use of such procedures? *What are the opportunities and challenges associated with achieving this?*

This is an on-going area of work for the RSPCA and we welcome invitations to visit establishments, examples of further case studies or suggestions for topics or procedures that could be addressed. Please contact elliot.lilley@rspca.org.uk

Refinements for SOD1 mice

Dominic J Wells and Hannah Kaneb,
Royal Veterinary College

The SOD1^{G93A} mouse is a frequently used model of amyotrophic lateral sclerosis (ALS), a form of motor neuron disease. ALS is a rapidly progressive, fatal disease commonly diagnosed at roughly 50 years of age, following which survival time is commonly just a further two to four years. Some 90% of cases are sporadic and the rest are familial. Approximately 20% of familial cases are caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Current treatments have only a very limited impact on survival and so the SOD1^{G93A} mouse is commonly used to test potential therapies.

The progression of motor neuron loss and development of clinical signs in these mice is very predictable. Treatments can be applied at various stages, but arguably the most relevant studies for translation to the clinic are those in which the treatment starts at the time of early clinical signs and delays the progression of the disease. This unfortunately means that the mice will exhibit progressive clinical signs during these studies and can ultimately experience severe suffering.

We take this very seriously and have set up a Standard Operating Procedure (SOP) for survival studies with SOD1 mice, with the aim of alleviating symptoms and refining housing, husbandry and endpoints so as to minimise suffering. All investigators and animal technologists have a copy of the SOP, which is summarised in Table 2. Some of the refinements are good practice for any type of study, but are especially important when striving to ameliorate severe procedures. We are aware that environmental enrichment can modify disease progression[12], but this is addressed by carefully ensuring that equal enrichment is provided to all mice.

In addition to the kinds of refinement outlined in the table, it is also very important to ensure that experiments are properly designed so as to use the optimum number of animals, minimise wastage and ensure that all variables are recognised and taken into account. Regrettably, however, there is evidence of

Husbandry	• Mice are genotyped before weaning and then placed directly into their experimental groups • Male and female mice are housed away from each other to avoid stress due to olfactory exposure • Gloves are changed and work surfaces cleaned between the handling of male and female mice • Some nesting material is carried over from the soiled to the clean cage when cage changing males, as this reduces aggression
Housing	• All cages contain ALPHA-dri® nesting material, 2 nestlets and 2 fun tunnels • Males are housed in groups of three, as this has been found to be the optimal group size to minimise fighting
Adaptations for disabled animals	• Fun tunnels are removed at 100 days, as mice may begin to show signs of hind limb weakness and paralysis and could become trapped or injured • Non-particulate litter and nesting material are provided, as animals may have difficulty grooming themselves • Cages are fitted with long sipper tubes and mash is provided daily (on a Petri dish lid in the corner of the cage) from 100 days • The length of the sipper tubes is carefully controlled to prevent contact with litter and nesting material, which can lead to leaks
Health checks and veterinary care	• General health and the righting reflex are checked twice daily from 100 days or earlier if motor problems are apparent • If mice have eye problems, the eyes are cleaned twice daily with sterile saline and lubricating drops applied (Tears Naturale®, Alcon) • If mice are dehydrated, they are given an i.p. injection of sterile saline (100 ml/kg/24 hours, which works out as 150-200 microlitres every 2 hours) and closely monitored
Experimental protocol	• Early screening of motor function and muscle characteristics should be used to select which drugs to take through to survival studies to minimise the number of mice suffering extreme motor deficits[11]
Interventions and humane endpoints	• Mice are separated from their cage mates as soon as they show signs of hind limb paralysis or if healthier cage mates show them undue attention • Animals are humanely killed if they are unable to right themselves within 20 seconds of being placed on their sides. Any mouse taking longer than 15 seconds to right in the evening check is euthanased and one day added to their survival time

Table 2. Standard Operating Procedure for caring for SOD1 mice

suboptimal study design in most of the studies performed in the SOD1^{G93A} mouse[13]. This includes poor recognition of confounding variables such as non-ALS mortality, 'clustering' of littermates and imbalance of the sexes. Scott et al. (2008[13]) proposed clear guidelines for improving ALS study design, but the majority of post-2008 publications have failed to adopt this best practice. A clear and immediate improvement would be to adhere to these guidelines, thus avoiding animal wastage on misleading experiments[5].

Action points:

- If using or caring for SOD1 mice, review housing, husbandry and care, and the experimental protocol, using table 2.
- Use earlier timepoints when screening possible treatments wherever possible, to avoid progressing to survival studies unless there has been a positive finding.
- Adhere to the guidelines on study design in Scott et al. (2008[13]), and mention these in publications and presentations.
- If not working with SOD1 mice but involved with other severe procedures, consider whether the approach to reducing and refining ALS studies could be applied in your own protocols.

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[5] See also the discussion of experimental design and reporting in Rice, earlier in this report.

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