Report of the 2007 RSPCA/UFAW Rodent Welfare Group meeting

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Summary

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 Three Rs 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 impact on their wellbeing has been reviewed and refined.

Presentations at the 2007 meeting included refining procedures in breast cancer and gut pathogen research, refining studies involving telemetry and championing animal welfare in toxicology. Other speakers addressed the evaluation of nesting material for mice with neurological disorders, training in phenotyping and good practice for sourcing rodents from third countries.

The welfare implications of high dose oestrogen pellets causing urinary calculi in athymic nude mice

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Breast cancer models that use slow release oestrogen (E) pellets in mice are widely used in many key laboratories and are currently important in breast cancer research, but we have discovered some inherent health and welfare problems for the animals. Fortunately, it has been possible to reduce the impact of these adverse effects by modifying experimental protocols and ensuring that the mice are effectively monitored.

Breast cancer is the most common cancer in women, with over 40,000 cases diagnosed every year in the UK alone. As E is known to be the most potent stimulant for breast cancer growth, many current drug therapies aim to oppose the effects of E (e.g. tamoxifen) or prevent it from forming (e.g. aromatase inhibitors). Our studies use xenograft models generated in athymic nude mice to investigate the action of such E opposing drugs. The mice are ovariectomised and, after a recovery period of at least 7 days, implanted subcutaneously with slow release E-pellets. This maintains E at clinically relevant doses. Mice are then injected subcutaneously with human breast tumour cells, which take two to four weeks to form a tumour approximately 7 mm in diameter. The animals are then randomised to receive the various therapies under study.

During the course of our studies we observed that two to three weeks after implantation of the E-pellet, the mice became dehydrated and displayed bladder distension and skin discolouration. Affected animals appeared to drag their hindquarters on the substrate and sore areas were visible around the urethra and tail base. The welfare of our mice is very important to us and any animals believed to be suffering or developing complications such as hard masses in the abdomen were humanely killed.

Autopsies revealed urinary calculi, or struvite, composed of magnesium ammonium phosphate, which is also found in human bladder and kidney stones. It is mainly caused by infection with urease-producing bacteria such as Proteus spp. and is associated with abdominal surgery in the clinic. Microbial assessment showed that Proteus type bacteria were indeed present in affected animals, but only in those implanted with an E-pellet, irrespective of whether they had had surgery. We concluded that the E-pellet stimulated the normally commensal Proteus to proliferate and cause chemical changes in the urine, leading to the formation of struvite.

As a result of these side effects, we altered our project licence to include the formation of struvite as a possible adverse effect, ensured that animal care staff monitored animals with E-pellets very closely and obtained veterinary advice on observing clinical signs and setting humane endpoints. It is quite clear that there must be some level of pain suffered by affected animals and it is essential that discomfort, pain and distress are effectively detected and assessed.

To this end, we have developed a simple 6-point guide to detecting the presence of struvite in mice with E-pellets:

W weight loss, which is an early indicator and can occur quite suddenly;
A abdominal distension, as a result of fluid retention and swollen bladder;

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S skin colour changes;
H hydration is poor, leading to poor skin condition;
U urethra irritation, where animals drag their hindquarters on the substrate and skin irritation and reddening are apparent;
P palpation of lower abdomen reveals the presence of calculi.

This "WASHUP" system has proved to be easy to use and remember and has greatly helped us to refine our endpoints and reduce suffering.

There are a number of other options that we are considering to avoid welfare problems with E-pellets. We have successfully genetically modified some of our tumour cell lines so that they can be grown under androgen support when implanted into the mice, which avoids the need for E-pellets. We have also refined our procedures for this model so that the time taken for tumours to develop is reduced, in turn decreasing the incidence of urinary calculi. These modifications include removing the E-pellets early, using alternative methods of administering E or commencing the therapy regime earlier whenever possible. In addition, we constantly review the literature on struvites in humans to see whether any equivalent therapies can be used in animals. For example, antibiotic therapy has not been ruled out but any potential effects on tumour models and therapies would have to be carefully evaluated.

Refinements in the management and short- and long-term monitoring of pathogen-infected mice

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It has recently become necessary to move from using rats to mice in salmonellosis studies, for scientific reasons, but this has led to welfare problems because many mouse strains are highly susceptible to systemic infection. There are a number of different approaches that can be taken to reducing suffering and improving welfare in mouse models of salmonellosis.

A key area of interest at the Rowett Research Institute is the study of the roles of bacteria, including pathogens, in modulating gut development and function and long-term health. We have been conducting studies on Salmonella enteritidis and typhimurium and the role of virulence factors in modulating the early stages of infection for some years (virulence factors: molecules produced by a pathogen that specifically influence their host's function, thus allowing the pathogen to thrive). Most of this work was done in rats, where a self-limiting gastroenteritis-like disorder develops which is similar to the common form of infection in humans. However, the emphasis has recently moved to studying the role of the innate and adaptive immune system in controlling gut-bacterial interactions in the short and long term. This requires changing from the rat to a mouse model of salmonellosis, because the necessary immune reagents and probes are currently available for mice but not rats.

Although mice are widely used in the study of salmonellosis, the infection triggered in most mouse strains is atypical and lethal, in contrast to the rat. The standard strains used are Balb/c or C57Bl/6, which are highly susceptible to systemic infection. Oral or i.p. infection with Salmonella typhimurium leads to uncontrolled proliferation and bacteraemia. This resembles typhoid-like disease in humans but is atypical of the infections caused by S. enteritidis or S. typhimurium in most domestic animals and humans, raising doubts as to the appropriateness of the model. This is also a concern on animal welfare grounds, so we have set up a number of measures that we use whenever possible to reduce or avoid suffering.

These are listed below.

- Treating mice with antibiotics, so that they develop an enteritis-like disorder, which is more clinically relevant[1].
- Using less susceptible strains, such as C3H/HeN[2].
- Housing in pairs. We use male or female mice, aged 7 to 8 weeks and weighing at least 19 g. Besides the welfare benefits of avoiding single housing, we have found that there are implications for our experimental results. Singly housed mice do as well as pair-or group-housed mice if they are healthy or have a low level of infection, but singletons do far worse if they have significant infection. We assume that data are less likely to be applicable from singly housed mice, due to the physiological effects of isolation stress. If mice are left alone because a member of a pair has reached an endpoint, we re-house them with counterparts at the same stage of infection wherever possible.
- Supplying tunnel inserts in metabolism cages for short term studies (Fig. 1). This provides them with a secure nesting area and they keep the tunnels very clean so there are no problems with faeces or urine collection. It also helps with handling because the mice can be lifted out while they are in the tunnels.
- Providing powdered, semi-synthetic diets (used in short term studies) in the form of freeze-dried blocks, so that the mice still get to gnaw on their food.
- Not fasting the mice before afternoon dosing, as we have found that they eat little during the day. We withdraw food at around 11:00 and dose the mice at around 15:30.
- Avoiding gavage when carrying out oral dosing by adding the bacteria or bioactive compound to a carrier that the animals will eat voluntarily. This works even for anaerobic bacteria. Mice prefer agar or potato starch

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paste spiked with fish oil, whereas rats like jelly or chocolate drops. Intake is usually rapid (one to ten minutes) but can depend on the time of day in mice; we have found it best to dose them early in the evening. Alternatively, suckling or newly weaned mice are dosed by dropping very small volumes onto the mid/back of the tongue using a milk-based carrier and micropipette with a round-ended tip. This can take several minutes. Gavage is used only when a single, precise dose is needed.

- Housing mice in standard cages with solid floors for long term studies. Faeces and urine are collected during weighing, which does not allow fully quantitative measurements but still provides good information about trends in bacterial excretion.
- Implementing humane endpoints by weighing and health scoring mice twice daily throughout a study and having a set of endpoint criteria (based on the health score and weight change) that trigger immediate removal of an animal from study. Animals are allocated a health score based on Shu et al.[3] (Box 1). The endpoint is a score of 2 or above plus weight loss over 4 successive weighings.

Effects of environmental enrichment on nesting behaviour and weight loss in a mouse model of Huntington's disease

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Rodents with neurological disorders may have difficulty in manipulating some nesting materials, and their ability to build nests can also deteriorate in the case of some progressive diseases. Setting up studies to evaluate the type of nesting materials that particular strains prefer can provide welfare benefits, in that (i) animals will be able to create a more comfortable environment and (ii) health and welfare monitoring will be enhanced if nest building ability is taken into account.

Huntington's disease (HD) is a progressive, inherited neurodegenerative disorder for which there is currently no treatment. It is characterised by movement abnormalities (a chorea with uncontrollable jerking and writhing movements), cognitive impairment and emotional disturbance. There are ten different mouse models of HD. One of the models that we use is the R6/2 strain, which shows a progressive neurological phenotype with abnormalities in motor function apparent from 5 weeks and cognitive deficits from 3.5 weeks. R6/2 mice show a progressive weight loss from 9 to 10 weeks and usually die aged between 16 and 20 weeks.

We were interested in studying the effects of environmental enrichment on nest building behaviour in R6/2 mice. Enrichment has been found to modulate the course of phenotype progression in several animal models of neurological disorders including Alzheimer's disease, Parkinson's disease and HD, and has been shown to slow disease progression in R6/2 mice[4,5]. Environmental stimulation for humans with HD, in the form of "remotivation therapy", has also been shown to improve functioning[6].

There are a number of housing refinements that we provide for our R6/2 mice to help them to cope with their disabling phenotype. They can find it difficult to reach the hard pellets in the hopper and drink from standard water bottles, so we give them supplementary feed in the form of mash made from 100 g dry food soaked in 230 ml tap water[7]. We also provide lower water spouts. Mixed phenotype housing, where R6/2 mice are housed with wild-type cage mates, has also proven to increase body weight and survival. The wild-type mice groom and rest with the R6/2 mice, presumably providing both comfort and stimulation.

R6/2 mice are already provided with paper wool nesting material as standard, but we wanted to investigate nest building behaviour in more detail. We conducted a study in three parts to assess; (i) nesting behaviour in R6/2 mice, (ii) the effect of different nesting materials on nest quality and mouse body weight and (iii) the effect of increased environmental temperature on weight loss. Part (iii) relates to the decline in body weight from 10 to 12 weeks in R6/2s; we wondered whether warmer ambient temperatures would result in a decrease in weight loss. The study will be written up for publication in full elsewhere and is summarised below.

Nesting behaviour

Groups of 5 mice were placed in clean cages with corn cob substrate and 10 g paper wool bedding shaped into a tight ball. A red perspex "igloo" house was placed in half of the cages. We used sixteen groups of male mice aged 12 to 14 weeks (8 wild-type and 8 R6/2) and eight groups of female mice aged 14 weeks (4 wild type and 4 R6/2). Nest building behaviour was quantified at 30 minutes, one hour and 18 hours after the mice were placed in the cages. The nests were scored as follows; 0 = nesting material unmodified, 1 = flat nest with no assembled walls, 2 = shallow nest lacking fully-formed walls, 3 = nest with well-developed walls and 4 = "coccoon" nest with partial or complete roof. The height of each nest was also measured after 18 hours. House and/or nest use was quantified by counting the number of mice in the house or nest (where present) at each of the time points.

All of the mice built nests, but there were some differences in nest quality. Wild type (WT) females had built better quality nests than WT males and R6/2 males at 18 hours ($P < 0.05$). R6/2 females were slower to begin nest building, but there was no difference in their nest quality scores at 18 hours when compared with WT females. In females, nest height was not influenced

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by the presence of a house, but both WT and R6/2 males built higher walled nests when housed without one. At 18 hours, all of the female WT and R6/2 mice were using their nests and houses and so were most of the male mice, although fewer R6/2 males with igloos were inside them. To conclude, although we know that nest building ability deteriorates with time in R6/2 mice, the key difference at 12 to 14 weeks lies in the females' latency to start building.

Different nesting materials

Groups of 10 female R6/2 mice, aged 10 weeks, were assigned to three treatments; (i) paper wool, (ii) sizzle nest or (iii) wood wool. Nest building was assessed daily by scoring nest quality and height as above, then calculating a weekly average score. Body weight was recorded twice weekly until the study ended at 22 weeks.

The mice with paper wool became more competent at nest building but could not form a grade 4 nest. They could not build a nest with walls at 22 weeks. None of the mice could build grade 4 nests with wood wool; the best attempt was a doughnut-shaped grade 3. Later stage R6/2 mice were incapable of building adequate nests with wood wool at all. Interestingly, the mice became better at building with sizzle nest as they got older.

Changes in the progressive deterioration in body weight observed in R6/2 mice were also dependent on the nesting material used ($P < 0.001$). All the mice gained weight normally up to approximately 15 weeks, then their weights remained relatively constant between 15 and 18 weeks (17 weeks in the case of the wood wool group). All of the mice then began to lose weight. The group with the wood wool lost most weight by the end of the study and the group with the paper wool lost the least. We concluded that sizzle nest should be the nesting material of choice for mice with neurological disorders and that careful consideration should be given to the use of wood wool for transgenic mice. The study also highlighted the importance of evaluating different nesting materials for different strains.

Increased environmental temperature

A heated chamber was constructed with clear, perspex walls secured to a metal floor with a water inlet and outlet. The chamber was placed in a standard mouse cage and connected to a water bath that maintained its floor at a constant 37 °C. Mice were housed in the cage containing the chamber in groups of 7 and were free to choose whether or not they entered the chamber. A control group ($N = 7$) was housed in a standard cage with a red perspex igloo. All mice were weighed twice weekly between 9 and 20 weeks.

Increasing the environmental temperature did not have an effect on delaying weight loss in R6/2 mice. The R6/2 mice preferred to nest in the heated chamber, but this had a deleterious effect on their survival. In part, this may have been because by staying in the chamber, they did not engage in normal behaviour in their home cage. On this basis, it appears to be better to allow R6/2 mice to create their own microenvironment by providing appropriate nesting material than to supply supplementary heating.

New insights using telemetry: refining endpoints and the value of group housed data

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Two key welfare issues associated with the use of biotelemetry are implant size and the practice of singly housing animals to avoid "cross talk" between devices that transmit on the same frequency. Telemetry device design is a rapidly developing field and there are a number of new developments in these areas that can improve the welfare of animals used in biotelemetry studies.

Biotelemetry provides the ability to monitor key physiological parameters such as core body temperature (Tc) in unrestrained subjects, which has widespread implications for improving scientific validity and providing objective measures of welfare. At present, the physiological burden associated with biotelemetry precludes its routine use in small animals for measuring parameters such as Tc, although temperature modulation of individuals and their social group is arguably of particular importance in behaviour and welfare studies.

Also, traditional biotelemetry systems are generally only able to monitor one animal at a time within a given arena due to "cross-talk" between devices transmitting on the same frequency. This can present a dilemma because, while single housing for mice should be avoided, there is also an imperative to maximise the data collected from all animals. Possible solutions are the "buddy" system, where animals are pair housed and only one individual is implanted[8], but an ideal biotelemetry system would be able to record from all animals in an arena. From an animal welfare aspect, devices should also be as small and light as possible, require less power (therefore a smaller, lighter battery), be able to monitor multiple parameters and have decreased surgical requirements[9].

A recent development in biotelemetry technology, the Remo 400 series, enables Tc to be continuously and remotely monitored in up to 16 group-housed individuals, without "cross talk" (www.remotechnologies.com). The device weighs under 1 g because it has no battery and is suitable for mice and larger animals. Body temperature follows a stable circadian rhythm by the third day following implantation surgery. Preliminary studies suggest that this time to recovery of circadian rhythm is shorter using these implants when com-

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pared to other, heavier devices[10]. Also, Tc measured in group housed mice is significantly higher, during both the light and dark phases, than that described previously in singly housed animals maintained under similar environmental conditions.

Previously unobtainable information on multiple animals within a social group can now be recorded and social species taking part in certain studies involving biotelemetry can now be maintained in an appropriate setting, gaining maximum formation from all animals [11,12,13,14].

Biotelemetry can be especially useful when studying infectious diseases, particularly in bio-containment, where the requirement for minimal human contact can create problems with monitoring disease progression and implementing humane endpoints. Telemetry has been successfully used to these ends in studies involving the characterisation of the response to toxic challenge and the assessment of vaccine efficacy. In projects such as these, it has been found that changes in Tc can occur before behavioural changes or clinical signs can be detected by simple observation. This can provide objective data to enable the refinement of humane endpoints. It can also detect the onset of infection, so that animals can be monitored more closely or given additional care. For example, using this telemetry technology, Tc changes following disease challenge have been found some 11 hours before any behavioural changes and 13.5 hours before the onset of clinical signs of disease[14]. Other studies have now described Tc response to viral infection in rodents in their normal social environment[11]. This is particularly significant when considering the differences in thermoregulation observed between group and singly housed mice.

In conclusion, advances in biotelemetry technology have clear implications for the Three Rs in relation to reduced device burden, the potential to group house and the possibility of reducing animal numbers due to better quality data. This technology can also be used to gain better insights into animal behaviour, refine humane endpoints and maximise the information obtained from each individual, making every single animal count.

Learning genetically altered mouse phenotype testing: training at the German Mouse Clinic

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As the use of genetically altered (GA) mice continues to increase, it is essential that all relevant staff are adequately trained in phenotyping and welfare assessment so that both expected and unexpected adverse effects can be effectively detected, assessed and alleviated.

For example, Cancer Research UK (CRUK) has over 300 GA mouse lines produced in house, with a total of over 500 crosses. Of these lines, 60 are known to have adverse phenotypes that affect the welfare of the mouse. Many more are suspected to have mild, moderate or neurological/behavioural phenotypes that have not been fully identified to date, yet may still impact on welfare.

Projects such as EUMORPHIA (www.eumorphia.org) and Europe-wide phenome projects have been catalytic in the increased popularity of mouse passport systems. Imminent plans for the expansion of CRUK's main mouse facility mean that in house production is expected to more than double. At least 60 lines are already known to have adverse phenotypes, and we are expecting to encounter more as our facility expands, so the need for phenotype identification and a mouse passport scheme at CRUK has become increasingly apparent. Accuracy and standardisation of phenotype testing will be key to their success. The specialized training required to perform such tests is often out of the realms of standard husbandry and technical training given to technicians.

Therefore, I embarked on a one month intensive training and work experience placement at the German Mouse Clinic (GMC) (www.mouseclinic.de) in order to gain the knowledge and technical skills required to perform standardised phenotype tests through to tertiary level. The GMC opened in 2002 and has a purpose-built phenotyping centre with a high-throughput system that evaluates a number of parameters including pathology, behaviour, neurology, morphology, clinical chemistry and cartilage and bone formation. Training consisted of phenotyping theory, practical work experience, lectures and discussions, data handling and statistics and the use of videos. We had hands on experience performing phenotyping tests, including with strains that had very unusual phenotypes. (During the discussion, it was pointed out that a UK training course is also available at the Medical Research Council's Mary Lyon Centre, Harwell; www.mlc.har.mrc.ac.uk)

The training and knowledge that I gained from the work experience placement will be used to launch a mouse phenotype passport system at CRUK. It is hoped that the passport system will bring about many welfare advances and bring to light any previously unknown phenotypes that affect mouse welfare. In particular, it should help to identify and record strain-specific needs with respect to handling, husbandry and general care, improve veterinary care and establish specific humane endpoints for adverse phenotypes.

Sourcing rodents from overseas

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Refinement includes the entire birth to death experience of every animal, including breeding and sourcing. Most animals used for research and testing in the UK are bred within the UK. However, if the spe-

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cies, strain or type of animal is not available, they may be imported from abroad. There are a number of measures that establishments can take to help ensure the welfare of imported animals before they reach their end destination.

In the case of rodents, conventional animals generally come from well known breeders, but it is not known how much information relevant to housing and husbandry is routinely collected by importers. There are many other potential sources of genetically altered animals and it is difficult – if not impossible – for the UK Home Office Inspectorate to influence them or even know who they all are (the Home Office Inspectorate implements the law regulating animal use in the UK, the Animals (Scientific Procedures) Act 1986).

In view of this, the Housing and Husbandry Sub-Committee of the Animal Procedures Committee (APC; www.apc.gov.uk) has published a document that reviews current practice and provides additional recommendations for importing animals that should be obtained from a designated breeding establishment under UK legislation[15].

The APC report recommends that importers and users should continue to collect data on the health status of imported animals. It also recommends that they should seek information from the supplier on the welfare and standards of housing and husbandry for all imported species, including rodents, that are listed in Schedule 2 of the UK Animals (Scientific Procedures) Act 1986. (This Schedule lists species that may be obtained only from designated breeding or supplying establishments.) Some researchers already exceed the current minimum regulatory requirements for imported animals, by obtaining information on animal welfare and husbandry conditions from their overseas suppliers. The APC Sub-committee commended this as good practice.

As a minimum, data should be collected on health status, any health issues, social housing, provision of enrichment and whether the supplying institution meets (or exceeds) relevant national or professional standards. Ideally, importers and users should visit the supplier or look at photographs or videos to satisfy themselves that appropriate standards are being met. The information can be judged against guidelines such as the current UK husbandry standards for breeders[16] or the recent revision of Appendix A to European Convention ETS123[17]. Alternatively, institutions may prefer to use their own guidelines for good practice, if these improve upon the relevant legal minimum standards[18].

The Committee recommends that institutional Ethical Review Processes (ERPs, which review the implementation of the Three Rs at a local level and are mandatory in UK establishments) should ensure that mechanisms are in place "to monitor and record health and previous housing or husbandry issues that could affect the welfare of imported animals and the quality of science derived from them". The Committee also recommends that "the aggregated information collected by ERPs should be reviewed at a National level within two years". A key aim of the review would be to check that the process collecting and reviewing data from overseas suppliers is robust enough to ensure good health and welfare, and to determine whether any revisions may be necessary.

The full APC report is available at http://www.apc.gov.uk/reference/2007-0404-web-version-standards.pdf

Championing animal welfare in toxicology

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When using rodents in regulatory studies, there is much that can be done to promote the implementation of the Three Rs among clients and regulatory bodies. I am currently Section Manager for the Rodent Division at Huntingdon Life Sciences. I have worked in contract and pharmaceutical research since 1987 and the following is based on my experiences as Facility Manager within a barrier facility with thirty animal rooms housing Specific Pathogen Free (SPF) rodents. Studies are carried out predominantly under a toxicology and oncology project licence, involving acute, neurological and reproductive toxicology through to lifetime carcinogenicity studies in rats and mice.

I have encountered a number of issues relating to animal welfare and environmental enrichment over the last 20 years, and we have been able to make significant progress in ensuring that the Three Rs are realised and that animal welfare and enrichment is prioritised. My staff have been able to collect significant data over a five-year period that allows quantification of some very tangible benefits. Three of the areas in which we have helped to introduce positive change are set out below.

"Spare" animals and effective animal usage

We aim to reduce the number of "spare" animals ordered and we record all instances of no-use in order to promote alternative usage. Data on "spares" and no-use is reported to the ERP quarterly. The number of "spare" animals not used fell to zero once we started having to report to the ERP, so it has been very influential in reducing wastage. Animals surplus to study requirements are now used for other purposes, e.g. to provide blood products for equipment validation or to provide tissues for training in necropsy and histology. Some are released from the Act and re-homed as companion animals.

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Individual housing

Group housing is our standard; five rats per cage or trios for mice. Any requirement for single housing must be authorised by the Named Veterinary Surgeon and Named Animal Care and Welfare Officer (NVS and NACWO; two posts required under UK law) before studies begin. There are some exceptions on veterinary or welfare grounds, such as some surgically prepared animals, dermal studies and male mice of specific strains. However, some clients have traditional preferences for single housing. We always challenge this and encourage them to comply with our welfare policy; they often agree to group housing for their animals. We have also challenged the requirement for single housing in the US Food and Drug Administration "Red Book" regulations for food additive tests, but unfortunately unsuccessfully to date. We also have to report the percentage of animals individually housed to the ERP and, again, the ERP has been influential in increasing accountability and bringing the numbers of singly housed animals down (Fig. 2).

Figure 1. Tunnel insert for use in metabolism cages

Environmental enrichment

Welfare issues confronted in rodent toxicology mainly relate to ageing animals, boredom and stereotypic behaviour. Animal technicians are encouraged to provide and research ideas to tackle all of these issues, such as environmental enrichment, and all such initiatives are reported to the ERP quarterly.

There may be issues associated with providing some types of enrichment on toxicology studies, but these can usually be overcome. For example, there are concerns about the lead content in cardboard "fun tunnels", especially as rodents chew and eat them. We use perspex "fun tunnels" instead, which come with a Certificate of Analysis, do not have a high lead content and are also autoclavable and reusable. We use perspex houses and "igloos" for our rodents.

Box 1. Health score system for Salmonella studies3

1. Bright eyed, alert, smooth coat, inquisitive
2. Fur slightly ruffled but active and alert
3. Ruffled/clumped fur, reduced activity, hyperventilates
4. Hunched and sleepy, fur clumped, little interest
   Hunched, very sleepy, non-responsive, cold

Keeping up with challenges

We are committed to pushing the boundaries and championing welfare initiatives, but there are some challenges that require due consideration. Our ongoing challenges are:

- Trialling new products to ensure that enrichment does not introduce other variables that may compromise study integrity
- Coping with clients' requests for novel techniques or procedures
- Clients' differing receptiveness to environmental enrichment
- Continued commitment to the review of current techniques and procedures so that refinements are implemented wherever possible
- Continuous review of Certificates of Analysis

We involve and inform Business Development with respect to all of the above processes and we educate everyone about our

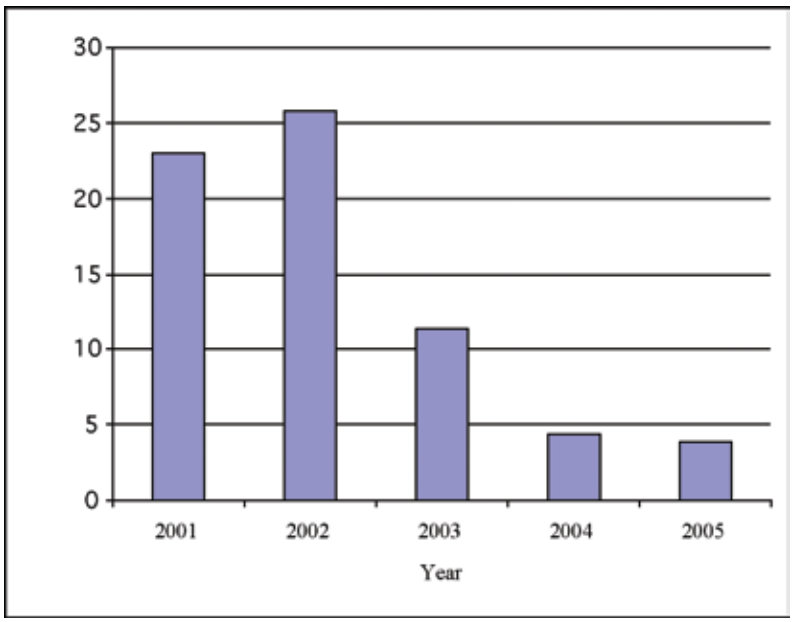

Figure 2. Decrease in the percentage of animals individually housed in a rodent toxicology facility following reporting to the ERP

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welfare policy and the reasons behind it. All NACWOs must make a presentation to the ERP every year about our welfare initiatives and refinements to husbandry and procedures. In this way we can all work together to promote better standards for animals in toxicology.

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