

TECH-2-TECH

Haven't the time to write a paper but want to get something published? Then read on!

This section offers readers the opportunity to submit informal contributions about any aspects of animal technology. Comments, observations, descriptions of new or refined techniques, new products or equipment, old products or equipment adapted to new use, any subject that may be useful to technicians in other institutions. Submissions can be presented as technical notes and do not need to be structured and can be as short or as long as is necessary. Accompanying illustrations and/or photos should be high resolution.

NB. Descriptions of new products or equipment submitted by manufacturers are welcome but should be a factual account of the product. However, the Editorial Board gives no warranty as to the accuracy or fitness for purpose of the product.

Report of the 2008 RSPCA/UFAW Rodent Welfare Group meeting

BARNEY REED, BSc (Hons), MSc,¹ PENNY HAWKINS, BSc, PhD (Secretary),¹ PROFESSOR STEPHEN HARRIS,² KATJA VAN DRIEL, BSc, MSc,³ PROFESSOR JANE HURST,⁴ DEBORAH DORE, BSc (Hons), PGCE (FAHE), PhD,⁵ NGAIRE DENNISON MA, VetMB, MRCVS,⁶ ROBERT DEACON,⁷ MAGGY JENNINGS, BSc, PhD¹ and ROBERT HUBRECHT, BSc, PhD⁸

¹ Research Animals Department – Science Group, RSPCA, Wilberforce Way, Southwater, West Sussex, RH13 9RS, UK.

² School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK.

³ Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK.

⁴ Mammalian Behaviour & Evolution Group, Department of Veterinary Preclinical Science, University of Liverpool, Leahurst Campus, Neston CH64 7TE, UK.

⁵ Deborah Dore, Rodbaston College, Penkridge, Staffordshire, ST19 5PH, UK.

⁶ Animals (Scientific Procedures) Division, Home Office, P.O. Box 6779, Dundee DD1 9WW, UK.

⁷ Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford, OX1 3UD, UK.

⁸ UFAW, The Old School, Brewhouse Hill, Wheathampstead, Hertfordshire, AL4 8AN, UK.

Correspondence should be addressed to Penny Hawkins (phawkins@rspca.org.uk)

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 impact on their wellbeing has been reviewed and refined.

The main themes of the 2008 meeting were:

- special considerations regarding the use and care of wild rodents in research and testing
- applying what is known about wild rodent behaviour

to inform the provisions made to safeguard welfare – both for them and for laboratory bred rodents more generally

In addition, there was a discussion session relating to the classification of clinical signs relevant to mild, moderate and substantial suffering in rodents.

The behaviour of wild rodents

Stephen Harris

(University of Bristol, UK)

There are 2277 species of rodent, which account for around 40% of known mammal species. Social systems vary from solitary and nocturnal, to complex and eusocial. Even within a species, behaviour can be highly flexible. The density at which the animals live is a key factor that impacts on behaviour, disease and stress levels.

Relatively few species of rodents are kept in laboratories, the main types being mice (particularly *Mus*), rats (particularly *Rattus*), hamsters, gerbils and guinea pigs. Where these animals have been kept over many generations in captivity, selection pressures have been towards tameness, reduced habitat selection, reduced mate choice, agonistic behaviour, reduced spatial needs, lower breeding success and juvenile characteristics.

For many reasons, wild-living individuals may also be taken into a captive environment. The behaviours of these undomesticated animals can be very different. The most common wild rodents used in British labs are brown rats, house mice and wood mice. Some facts about the natural behaviour of these species follow:

Brown rat

Habitat

Highly adaptable. There are generally few competing species. Usually dig burrows and may swap these daily. Nests underground. Caches food underground.

Social behaviour

Tend to live in large colonies which are aggregations of smaller units. Each unit has a mating pair or harem that defends the territory around the burrow. At high densities, dominance systems operate, with high-ranking males and their harems attempting to occupy the most favourable positions and excluding others from food sources.

Movement

The distances travelled depend on food distribution. The longest single journey recorded is 3.3km in a night, but in food stores the average observed has been

around 65 metres. Individuals trot along familiar runs and are seen to amble when searching for food. This species both swims and climbs well.

Communication

Both auditory and olfactory communication are important in a range of social interactions. Sniffing and grooming are also important. Can hear sounds up to 110kHz. Most vocalisations are above 20kHz. May call 50-150 times a minute.

Breeding

Promiscuous mating system. At low densities one male mates with multiple females (polygynous). At high densities there is a tendency towards multiple males mating with multiple females (polygynandry). Females often exhibit post-partum oestrus and pregnancy may be extended when lactating.

House mouse

Habitat

Not obligate commensal. Habitats range from coral atolls to near Antarctic conditions, deserts and the London underground tube system. Does not compete with wood mouse and is now rare in British rural habitats.

Social behaviour

Very flexible. In commensal situations, home range is small with mixed sex groups of single dominant male, one or more breeding females and variable number of subordinates. In feral situations home range is much larger and difficult to defend so overlap extensively. Densities can exceed 10,000 per 100 cubic metres in ricks.

Breeding

Chemical signals regulate individual female reproductive success. Dominant males deposit many more scent marks than subordinate males or females. Females in same breeding group usually pool litters in communal nest and nurse pups indiscriminately.

Wood mouse

Habitat

Highly adaptable and opportunistic. Primarily woodland (where they climb trees and bushes) but virtually any rural habitat. Male home ranges extend up to 40,000 square meters. Males may travel long distances during the mating season.

Social behaviour

In winter a loose organisation exists with communal nesting of both sexes.

Breeding

During the breeding season, males will develop a

dominance hierarchy and will compete to mate polygynously with promiscuous females. This leads to multiple paternity litters.

Black rats, spiny mice, deer mice, yellow-necked mice, harvest mice, bank voles, field voles, water voles, red and grey squirrels, hazel dormice and edible dormice may, for example, also be used. Wild rodents are mainly used in toxicology, behavioural studies (e.g. sperm competition, cognition, scent marking, mate choice, thermoregulation), immunology, parasitology, trapping and immunocontraception studies.

Whether using a rodent taken directly from the wild (or indeed one who is the product of many generations of captive breeding) it is very important to acknowledge that keeping animals in captivity affects both physiology and behaviour. Although behavioural systems of rodents can be very flexible it may be hard to provide long-term housing for most/all species without influencing behaviour. The natural behaviours of the species must be considered (along with the extent to which performance of these behaviours are important to the individual) in order to establish appropriate provisions for their care and welfare.

Welfare of wild rodents used in research

Katja Van Driel, BSc, MSc

(Central Science Laboratory, UK)

Compared to housing 'conventional' laboratory rodents, a number of additional issues need to be taken into account when keeping wild rodents in a laboratory. This is important to ensure both the welfare of the animal, and the welfare of the caretaker.

Welfare should be safeguarded right from the beginning of a study, starting with capture from the wild. Both capture and transport to the laboratory are stressful conditions that also carry additional legal requirements under the Animal Welfare Act 2006, the Animals (Scientific Procedures) Act 1986, and others depending on the species.

Once in the laboratory it is important to remember that the animals are entirely dependent on humans for meeting their welfare needs. Suitable housing and environmental factors should be assessed specific to the species. For example, conventional cages used for housing rodents are generally unsuitable for wild rats, and consideration should be given to the climatic conditions in the wild from which the animal was taken (e.g. capture taking place during winter versus summer).

Physical contact between humans and animals should be minimised. As such, pre-planning is essential when

needing to handle wild rodents. Different species require different methods and confident handling is required at all times.

Knowledge of the wild animal's normal behaviour in a captive situation (so not necessarily its normal behaviour in the wild), its appearance, physiological and anatomical characteristics is essential to be able to monitor the animal throughout a study. This is particularly because wild animals especially tend to appear stoic and relatively impassive when in pain or discomfort.



Figure 1. Technique used to handle a wild mouse.

(Photo: Central Science Laboratory)

Regular monitoring (at least daily) is required, but attention must be given to the psychological effects of handling and a suitable balance of intervention must be found per species and trial.

Finally, where it is deemed inappropriate following completion of the study to release animals back to the wild, a suitable method for euthanasia must be selected involving the minimum amount of pain and distress as well as maximum safety for the caretaker.

In summary, working with wild rodents requires an additional set of skills to those needed when working with conventional laboratory animals, though some handling methods in particular can also be used for conventional rodents.

Born to be wild – the consequences for house mouse husbandry and welfare

Jane Hurst

(University of Liverpool, UK)

The house mouse (*Mus musculus domesticus*) is a

human commensal that has adapted to exploit built environments where there are concentrated food resources, and where physical structures provide protection from predators, humans and poor weather conditions. Although highly adaptable to a wide range of physical conditions and diets, their key to survival is to remain hidden and elusive to human capture or other control measures. Lighting and cover are thus extremely important in influencing behaviour and the stress response on encountering people. The amount of space provided in captivity is rather less important than the quality of the space.

Lighting

All mice are nocturnal. This means that both the checking of animals, and many experimental procedures, are better carried out during the normal active dark period if possible. This will require establishments to implement a 'reverse lighting' regime. Observation and handling by humans in this context may be facilitated by the use of dim red lighting.

Cover

As prey animals, shelters and objects to break up open spaces within cages and enclosures are very important to mice and some form of cover should always be provided. The provision of ground cover will considerably increase mouse activity and their use of space. Objects and other vertical surfaces may also be used as pathways. Nest material, tubes (cardboard, transparent acrylic) and shelters (including those that attach to the cage lid where mice can easily be inspected) are very well used (Fig 2).



Figure 2. Providing shelter for mice is important.
(Photo: Jane Hurst)

Human interaction

Despite generations of captive breeding that have favoured a number of traits associated with domestication, laboratory mice are still not inherently 'tame' to human contact. However, training can have a

major effect on reducing stress responses to appropriate handling and other disturbances. It is important to familiarise mice (laboratory or wild) with procedures and new equipment and follow a consistent approach. Should mice 'freeze' or adopt the 'stretch attend' posture, then this is good evidence of anxiety though the considerable reduction in 'panic' behaviour of laboratory mice (compared to wild mice) does not in itself necessarily indicate lowered anxiety.



Figure 3. Clear handling tunnel.
(Photo: Jane Hurst)

Wild mice should be handled indirectly as much as possible to reduce handling (Fig 3) stress, for example by allowing them to run into clear handling tunnels.

Experience and training have a substantial influence and this is likely to be a major factor underlying variability in behavioural and physiological responses observed in mice both within and between laboratories.

Health

The incidence of parasites and diseases are surprisingly low in captive-bred mice, but lymphocytic choriomeningitis virus (LCMV) is a zoonotic infection that spreads very readily in captivity and requires an effective quarantine and screening programme¹.

Social grouping

Although well adapted for living in social groups, competition for breeding opportunities frequently leads to injurious or fatal aggression among group-housed males in cages and in most cases males beyond full sexual maturity need to be housed separately when housed in standard laboratory cages.

The derivation of today's populations of laboratory mice from a tiny gene pool has resulted in a subspecies hybrid with extremely reduced genetic variation within and between all of the classical laboratory strains². The consequences for social recognition and competitive behaviour are mostly positive with respect to laboratory welfare and management, but disturbance of a restricted social environment can lead to extreme responses.

Scent is extremely important to mice for learning information about others and is crucial for regulating appropriate social responses³. Use of scent allows for rapid recognition of individuals at a distance. To reduce immediate aggressive responses to 'unfamiliar' individuals, caretakers should avoid exposing mice (particularly males) to scents that stimulate competitive responses, and should familiarise them well with unfamiliar scents in the home cage prior to (re)introduction.

As there is also good evidence that mice can detect airborne alarm signals emitted (e.g. during culling), appropriate steps should be taken to negate this (e.g. by isolating animals currently being culled from others).

The behaviour and welfare of Egyptian Spiny Mice (*Acomys cahirinus*) in the domestic environment

Deborah Dore, BSc(Hons), PGCE(FAHE), PhD
(Rodbaston College and The University of Wolverhampton, UK)

Some rodent species are taken into captivity by humans as pets or for use in research and testing for example. Welfare can be compromised when a detailed understanding of the animal's physical and behavioural needs is lacking. A study involving 180 Egyptian Spiny Mice (*Acomys cahirinus cahirinus*) provides an example of work being undertaken to investigate one such species of the Muroidea superfamily.

Despite the popularity in many countries for keeping Spiny mice (*Acomys spp.*) as pets, there is still relatively little knowledge available relating to how they can be housed successfully. This study aims to help determine the appropriate housing, diet and social grouping necessary to maintain a good standard of care and welfare.

Physical environment

Glass aquarium tanks, with a minimum height of 45cms, were used successfully to house this species during the study. Tanks had a secure ventilated metallic mesh lid and a heat lamp, where required.

In the wild, most desert rodents favour a shrub microhabitat over open ground.⁴ Nocturnal rodents tend to seek cover to avoid predator detection using a topographically complex environment.⁵ Although *Acomys* species do not burrow themselves, they do use abandoned burrows such as those made by gerbils⁶ and so in captivity, artificial burrows should be provided.⁷ Burrows may also be used to aid thermoregulation (and

reduces water loss) but in captivity, where thermostats can be set to prevent temperatures falling below 19°C, this need appears to be reduced.

A substrate of bird sand was used as it is easy to manage, with regular sieving and removal of damp areas from the corners of the tank. Hollowed wooden logs and branches were provided to allow opportunities for elevation and, along with stones,⁸ are utilised as navigation posts. Mice were often observed to jump from one object to another and so avoid having to come into contact with the ground. Covered nesting areas, especially bird baskets, and hay bedding were readily utilised by the mice. If running wheels are provided for exercise they should be solid plastic, or ideally, solid mesh. This is because the tails of these mice can easily become detached if they are caught in traditional barred wheels.

Social grouping

These animals have highly complex social structures, which is atypical for nocturnal rodent species⁹. When housed in groups, individuals were rarely observed on their own. During the study, mice were successfully housed in mixed sex colonies of 20 to 50. Individuals frequently engaged in allogrooming. Some problems were encountered when regrouping adults (although juvenile females have been successfully added to an established adult female group using a neutral tank), and some aggression was observed when maintaining male-only groups larger than 7 individuals. However, when in family colonies, they are intolerant of intraspecific intruders. Even returning a family member after short separation can cause aggression due to the rapid re-establishment of dominance hierarchy.

Breeding occurred throughout the year in captivity, but when the colony reached its maximum capacity, breeding became inhibited. A dominant pair exists in breeding groups, with both males and females having social hierarchies that can act to suppress the breeding opportunities of subordinates. A dominant female encountering the offspring of a subordinate female may be aggressive towards them. Male and female infanticide has been observed¹⁰ but in this study, actual infanticide appeared extremely rare, with only one observed case in three years.

Behaviour

When given the opportunity, and in particular when housed in larger groups, these mice spent significantly more time on elevated structures than on the ground (Fig 4). Mice engaged in sporadic bouts of activity throughout the 24-hour period but were predominantly active during late evening, peaking just before midnight (when they were routinely fed). Whilst such an adaptation is used by many species in the wild to reduce the risk of predation, when food is scarce¹¹ or of low quality⁴ wild spiny mice

have been observed to actively forage throughout the 24-hour period. Mice were observed to be curious and responsive to human interaction, and were quick to adapt to routine schedules.



Figure 4. Egyptian spiny mice utilising elevated structure.
(Photo: Jacob Dore © 2008)



Figure 5. Egyptian spiny mouse drinking.
(Photo: Adam Dore © 2008)

Feeding

Feeding mainly took place nocturnally. Mice were fed on 25% rodent mix, 25% degu mix and 50% budgie/canary seed diet in ceramic bowls, and eagerly foraged for mealworms or dried insects. Coprophagy was observed during daylight hours. Mice showed favoured feeding times and diet, with a minority of certain individuals observed to hoard favourite foods. In the wild, snails are a favoured food (perhaps due to their water content) but in captivity these mice have been observed to prefer dried mealworms to live or re-hydrated ones.¹² As an adaptation to the xeric (lacking in moisture) environments in which these mice are found naturally, mice seldom urinate, and lactation is also limited.¹³ In captivity, these mice will readily use water bottles when fed a dry diet (Fig 5). Providing a false bottom to the plastic bottles prevented mice sitting on the top from chewing through them.

Conclusion

The growing trend for housing exotic rodents has often outstripped education on appropriate animal husbandry

methods. With specific regard to *Acomys* species, since the first observations on these animals as pets⁶ reported that these mice “attack humans... remain wild; interacting little with humans... living 2-3 years”, successful domestication over the last decade has meant these observations may no longer apply. The mice in this study were friendly, curious, and adaptive, and after three years, none have yet been lost to old age.

The use, care and accommodation of wild animals or less usual species in scientific procedures – an inspector’s view

Ngairé Dennison, MA, VETMB, MRCVS
(Home Office, UK)

Source of animals

Prior to the use of wild caught rodents (in scientific procedures), the justification for their use and any requirements for exemption from Schedule 2 of the Animals (Scientific Procedures) Act 1986 (which requires that all commonly used laboratory animals are purpose bred and obtained from an approved establishment)¹⁴ need to be considered and discussed with the Home Office Inspectorate, and any licence authorities required should be put in place. The local ethical review process also has an important role in considering the ethics of using unusual species or wild caught animals.

In the UK, capture of wild animals is considered a husbandry practice and is therefore exempt from ASPA controls though if any regulated procedures are to be undertaken at the site of capture, ASPA licence authorities require that this place of work be named (commonly these places are referred to as a Place Other than a Designated Establishment, or PODE). However, ethical considerations still apply.

Appendix A to European Convention ETS123¹⁵ states that humane methods should be used, with provisions in place for dealing with captured animals found to be injured or in poor health. There may be additional requirements under other legislation or codes relating to capture or supply of animals, such as CITES, the Wildlife and Countryside Act 1981, Nature Conservation (Scotland) Act 2004, Conservation (Natural Habitats) Regulations 1994; or their transport. Guidance on the transport of experimental animals is published in Laboratory Animals (2005) 39(1) 1-39. In the UK, administration relating to the issuing of relevant permits and licences for capture is generally handled by DEFRA, English Nature and Scottish Natural Heritage.

When taking any animal from the wild, special consideration must be given to acclimatisation and quarantine, and expert advice should be taken (e.g. from a Named Veterinary Surgeon) on the acceptability of health status and zoonotic risks.



Figure 6. Water vole – an example of a less usual species used.

Husbandry

Appropriate husbandry provisions are not only essential for good welfare of both wild caught and less commonly kept species, but are also required for reasons of good science. The most important single requirement in establishing conditions for the successful maintenance of wild species in captivity is to be properly informed of the biological and environmental needs of the species in its natural habitat.

Consideration needs to be given to temperature, humidity, breeding requirements, noise and other disturbances (e.g. handling), nutrition (which must be relevant to the physiological status of the individual animal), photoperiod, social hierarchies, provision of complex environments, and behavioural repertoire. The Home Office expectation is that standards set out in the Codes of Practice for rodents will be adhered to, but other parameters can be justified based on knowledge of the specific needs of the species. Information on such requirements should be obtained from those with relevant expertise for the species, and proposed husbandry systems should be agreed with the local Home Office Inspector at an early stage.

Social housing

Unless naturally solitary, animals should be housed in socially compatible, stable groups. Steps should be taken to acquire compatible individuals and subsequent disruption to these social groups should be minimised. Single housing is only acceptable where there are exceptional veterinary, welfare or scientific reasons. Where animals are singly housed, this should be for the minimum time necessary and visual, auditory, olfactory and tactile contact should be maintained wherever possible.



Figure 7. Housing social animals (like these gerbils) together is important.

Environmental complexity/enrichment

Sufficient space and adequate complexity should be provided to allow animals to express normal species-specific behaviours such as foraging, exercise and manipulative activities. Animals also benefit from having a degree of control or choice over their immediate environment.

Special care is needed for wild caught animals. Enclosures should be safe, easily cleaned (or disposable) and their design should incorporate 3D complexity in the form of shelves, sleeping structures/shelters, a solid lying area, tubes, climbing racks and wheels, as appropriate to the species. Litter, bedding and nesting materials should also be provided with consideration given to any specific needs (e.g. breeding animals). Enrichment programmes should be regularly reviewed and updated.

Temperature/humidity

When establishing protocols for temperature and humidity, where the standard Code of Practice is not appropriate, any effects on breeding performance, health of offspring and frequency of cage cleaning required must be considered.

Nutrition

In order to meet both the nutritional and behavioural needs of the animals, carers need to think carefully about what to provide, along with how to present it. This means considering, for example, the foraging behaviours of the animals, their age and physiological state, and how animals will access the diet.

Photoperiod

This may have possible effects on the animals relating to their weight, body composition, and reproductive performance.

Breeding

When seeking to breed animals, consideration should

be given to the natural social structure of the species (e.g. pairs, trios, harems, multiple males, hierarchies where only dominant animal/pair breed). In colonies involving only a small gene pool, inbreeding can lead to husbandry problems.

Noise/disturbance

This can be a particular issue for wild caught animals or for certain species and it may impact on the animals in various ways (e.g. may affect breeding performance). It may be beneficial to leave animals undisturbed for specific periods (e.g. first 48 hours after giving birth). Where animals cannot be checked daily for welfare reasons, this should be discussed with the local Home Office Inspector.

Fate of animals

This should be considered right from the outset. Discharge of animals from the Act at the end of procedures, allowing return to the wild, requires authorisation by the Home Office. Release of animals at the end of a study may have an impact on both how animals are housed and handled and can have potential welfare issues with respect to the impact of the release on the animal itself, or others in the local area.

Sources of information

- Codes of Practice and published general guidelines
- Home Office Inspectorate
- Experts
- Named vets
- Technicians/keepers/care staff with experience of keeping the species
- Literature reviews on specific species
- Other groups with experience of caring for the species in an experimental setting
- Internet (but view information with a degree of caution)

Use of ethologically based behavioural tests to characterise rodent welfare and phenotype mutant mice

Rob Deacon

(University of Oxford, UK)

Mice have a rich repertoire of everyday activities. Several protocols have been developed to measure the parameters of these “activities of daily living” (ADL). Changes, and deterioration in ADL, are one of the first symptoms of Alzheimer’s disease and other central nervous system pathologies such as depression. Using mouse ADL, almost homologous models of such disorders can be created. The tests are generally simple and easy to perform. Specialised, expensive

apparatus is not required. Perhaps most importantly, mice do not appear to find such testing aversive. Indeed, their active display of such ADL without additional motivation suggests that they find these tests reinforcing.

These protocols could even be adapted to provide environmental enrichment in their home quarters. For example, it is recommended that the depth of bedding material in the home cage be increased to the maximum practical, as mice rapidly spontaneously dig if the depth of bedding is 5cm (the standard used in our digging protocol).

Performance on tests relating to burrowing, digging, marble burying, hoarding, cage edge walking, nesting (Fig 8), climbing and the tunnel maze was observed to be dramatically impaired by hippocampal lesions, which are one of the first histopathological landmarks of Alzheimer’s disease.

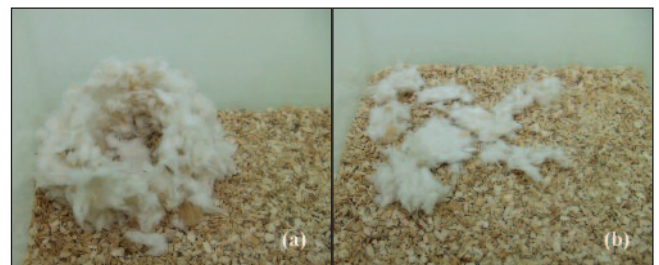


Figure 8. Normal nest-building activity (a) in mice is clearly impaired following lesions of the hippocampus (b).

These protocols are more fully described elsewhere.¹⁶⁻²²

Discussion Session: Is the FELASA list of clinical signs for rodents still valid?

In 1994, FELASA published a report on pain and distress in laboratory rodents and lagomorphs.²³ This included a table (Fig 9) produced by Buckwell²⁴ setting out common physical signs of ill-health recognized or reported in rodents at the time, which were considered to be associated with the mild, moderate and substantial categories set out in the Animals (Scientific Procedures) Act 1986. It was hoped the table could help researchers to think about the impact of their work on animals and consider how humane endpoints might better be defined, for example, as the sum of various signs listed in the table.

The table has been extremely helpful in grading severity and is still widely used (though sometimes in ways not originally intended by its authors). However, since the table was originally drawn up much has been learned about rodent behaviour. It is recognised that many behavioural signs of suffering can be extremely subtle.

Note: the first column has been added

	Mild	Moderate	Substantial
Body weight	Reduced weight gain	Weight loss of up to 20%	Weight loss greater than 25%
Food and water consumption	Food and water consumption 40-75% of normal for 72 h	Food and water consumption less than 40% of normal for 72 h	Food and water consumption less than 40% for 7 days, or anorexia (total inappetence) for 72 h
Piloerection	Partial piloerection	Staring coat - marked piloerection	Staring coat - marked piloerection - with other signs of dehydration such as skin tenting
Responsiveness	Subdued but responsive, animal shows normal provoked patterns of behaviour	Subdued animal shows subdued behaviour patterns even when provoked	Unresponsive to extraneous activity and provocation
Peer interaction	Interacts with peers	Little peer interaction	
Hunching	Hunched transiently especially after dosing	Hunched intermittently	Hunched persistently ('frozen')
Vocalization	Transient vocalization	Intermittent vocalization when provoked	'Distressed' - vocalization unprovoked
Oculo-nasal discharge	Oculo-nasal discharge transient (typically signs of chromodacryorrhoea in rodents)	Oculo-nasal discharge persistent	Oculo-nasal discharge - persistent and copious
Respiration	Normal respiration	Intermittent abnormal breathing pattern	Laboured respiration
Tremors	Transient tremors	Intermittent tremors	Persistent tremors
Convulsions	No convulsions	Intermittent convulsions	Persistent convulsions
Prostration	No prostration	Transient prostration (less than 1 h)	Prolonged prostration (more than 1 h)
Self-mutilation	No self-mutilation	No self-mutilation	Self-mutilation

Figure 9. Physical signs for rodents related to mild, moderate and substantial severity

There is now increased emphasis on looking at using the animals' interactions with their environment as welfare indicators, such as reduced nest building, gnawing on chew blocks, foraging for supplementary food in the litter and so on. Much too has also been learned about physiological signs of reduced health or wellbeing and people are utilising physiological indicators that may occur in the absence of any changes visible to the human observer.

Some people's views have also changed about particular definitions in terms of how they relate to different levels of suffering and in more general terms the issue of what actually constitutes mild, moderate and substantial suffering has been the recent subject of debate.²⁵

With this in mind, views were sought from Rodent Welfare Group members as to:

- i) whether the signs used in the table are descriptive enough;
- ii) whether the signs could be quantified more accurately;
- iii) whether the signs are appropriate for the level of severity they are describing.

Consideration of these issues is pertinent given possible future changes in the way the severity of animal suffering is reported in the UK.²⁶

The discussion highlighted that there is general agreement that the contents of the table provide a good

starting point for considering the impact of an experiment on an animal. However it is essential that the table is then tailored to the specific protocols to be used, and the species, strain and origin (wild versus lab bred) of animals involved. It was also agreed that adding some description to the clinical signs, could be beneficial.

Views were expressed that:

- The context in which animals were being observed was extremely important. For example, over what period of time did weight loss occur, and what was the starting weight of the animal?
- There may sometimes be conflict between signs.
- The contents of this table should not be directly extrapolated to other species. Indeed, there is a need even to tailor the tables separately for mice and for rats.
- In some cases, the signs need to be expanded e.g. water intake should also take into account 'increased' drinking.
- The signs would be viewed differently if observed in stock animals versus those involved in experiments.
- Possible new criteria or signs could include: gait, measurements involving urine/faeces, grinding of teeth or excessive chewing, rectal temperature, increased responsiveness to touch, exploratory behaviour (e.g. rearing), repetitive or stereotypic behaviour, grooming behaviour.
- Different emphasis is usually given to different signs within the table e.g. body weight loss is often viewed as one of the most important indicators.
- Since the table was first established, the generation of transgenic animals has escalated exponentially. Phenotype can have a considerable impact, both on what might be considered 'normal behaviour' and on an animal's ability to engage in certain behaviours.
- In order to properly assess the potential relevance of the clinical signs it is imperative that people are able to recognize what might be regarded as normal behaviour of the particular animals in their care.
- There is a degree of subjectivity involved in assessment, and this possible variability will increase where signs are not descriptive. For example, one person's 'transient' is another's 'intermittent'. This subjectivity may also then extend to considering what this means in terms of the animals' experiences e.g. some people may also feel that substantial suffering for ten minutes is better than moderate suffering across three days – others may feel the opposite is true.
- This table should be considered to be an evolving document, requiring regular review as new knowledge comes to light.

Subject to further consultation, future work to take account of these deliberations may occur during 2009. Please contact: research_animals@rspca.org.uk for further information.

Acknowledgements

The Group would like to thank all the speakers and everyone who attended and participated on the day.

Competing interests

The authors declare no competing financial interests.

References

- ¹ **Becker, S.D., Bennett, M., Stewart, J.P. and Hurst, J.L.** (2007). Serological survey of virus infection among wild house mice (*Mus domesticus*) in the UK. *Laboratory Animals* **41**, 229-238
- ² **Cheetham, S.A., Smith, A.L., Armstrong, S.D., Beynon, R.J. and Hurst, J.L.** (2008). Limited variation in the Major Urinary Proteins of laboratory mice. *Physiology & Behavior*, in press. Available online: doi:10.1016/j.physbeh.2008.10.005.
- ³ **Hurst, J.L.** (2005). Making sense of scents: reducing aggression and uncontrolled variation in laboratory mice. Available online at: www.nc3rs.org.uk/news.asp?id=164.
- ⁴ **Ylonen, H. and Brown, J.S.** (2007). Fear and the Foraging, Breeding and Sociality of Rodents in Wolff, J.O. & Sherman, P.W. (editors) *Rodent Societies; an Ecological and Evolutionary Perspective*. The University of Chicago Press, Chicago and London. Chapter 28: 330-335
- ⁵ **Nutt, K.J.** (2007). Socioecology of Rock Dwelling Rodents in Wolff, J.O. and Sherman, P.W. (editors) *Rodent Societies; an Ecological and Evolutionary Perspective*. The University of Chicago Press, Chicago and London. Chapter 35: 417
- ⁶ **Shargal, E., Kronfeld-Schor, N. and Dayon, T.** (2000). Population Biology and Spatial Relationships of co-existing Spiny Mice (*Acomys*) in Israel. *Journal of Mammalogy* **81**, 1046-1052
- ⁷ **Verhoef-Verhallen, E.** (1998). Encyclopaedia of Rabbits and Rodents. Rebo Productions Ltd. 75-77
- ⁸ **Eilam, D.** (2004). Locomotor Activity in Common Spiny Mice (*Acomys cahirinus*); The Effect of Light and Environmental Complexity. *BMC Ecology* **4**, 16
- ⁹ **Wolff, J.O. and Sherman, P.W.** (2007). (eds). *Rodent Societies; an Ecological and Evolutionary Perspective*. The University of Chicago Press, Chicago and London
- ¹⁰ **Porter, R.H. and Doane, H.M.** (1978). Studies of Maternal Behaviour in Spiny Mice (*Acomys cahirinus*) *Zeitschrift fur Tierpsychologie* **47**, 225-235
- ¹¹ **Elvert, R., Kronfeld, N., Dayan, T., Haim, A., Zisapel, N. and Heldmeier, G.** (1999). Telemetric Field Studies of Body Temperatures and Activity Rhythms of *Acomys russatus* and *Acomys cahirinus* in the Judean Desert of Israel. *Oecologia* **119**, 484-492
- ¹² **Dore, D. and Bruton, L.** (2007). Diet Selection of Egyptian Spiny Mice (*Acomys cahirinus*). Poster Presentation; RSPCA/UFAW Rodent Welfare Meeting (12th October) CRUK, Cambridge
- ¹³ **McGuire, B. and Bemis, W.E.** (2007). Parental Care in Wolff, J.O. and Sherman, P.W. (editors) *Rodent Societies; an Ecological and Evolutionary Perspective*. The University of Chicago Press, Chicago and London. Chapter 20: 231-242
- ¹⁴ **Home Office. Schedule 2** (2000). Animals to be obtained only from designated breeding or supplying establishments: in Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. The Stationery Office, London
- ¹⁵ **Council of Europe** (2006). Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No.123) – Guidelines for Accommodation and Care of Animals (Article 5 of the Convention). Council of Europe, Strasbourg
- ¹⁶ **Deacon, R.M.J. and Rawlins, J.N.P.** (2006). T-maze alternation in the rodent. *Nature Protocols* **1**, 7-12
- ¹⁷ **Deacon R.M.J.** (2006). Appetitive position discrimination in the T-maze. *Nature Protocols* **1**, 13-15
- ¹⁸ **Deacon R.M.J.** (2006). Burrowing in rodents: a sensitive method for detecting behavioural dysfunction. *Nature Protocols* **1**, 118-121
- ¹⁹ **Deacon R.M.J.** (2006). Digging and marble burying in mice: simple methods for *in vivo* identification of biological impacts. *Nature Protocols* **1**, 122-124
- ²⁰ **Deacon R.M.J.** (2006). Housing, husbandry and handling of rodents for behavioural experiments. *Nature Protocols* **1**, 936-946
- ²¹ **Deacon R.M.J.** (2006). Assessing nest building in mice. *Nature Protocols* **1**, 1117-1119
- ²² **Deacon R.M.J.** (2006). Assessing hoarding in mice. *Nature Protocols* **1**, 2828-2830
- ²³ **FELASA Working Group on Pain and Distress** (1994). Pain and distress in laboratory rodents and lagomorphs. *Laboratory Animals* **28**, 97-112 www.lal.org.uk/pdf/files/FelasaPain.pdf
- ²⁴ **Buckwell, A.** (1992). Limiting clinical signs appendices. Laboratory Animal Science Association – Winter Newsletter, 16-17
- ²⁵ **The Boyd Group/RSPCA.** (2004). Categorising the severity of scientific procedures on animals. RSPCA, Horsham
- ²⁶ **LASA/APC.** (2008). Final report of a LASA/APC Working Group to examine the feasibility of reporting data on the severity of scientific procedures on animals. Available online at: www.apc.gov.uk/reference/lasa_apc_final_report.pdf