Refining rodent husbandry: the mouse

Report of the Rodent Refinement Working Party

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Preface

Whenever animals are used in laboratories, minimizing any pain and distress (i.e. Refinement) should be as important an objective as achieving the experimental results. This is important both for humanitarian reasons and in order to satisfy broad legal principles, such as stated in the European Directive regarding the protection of animals used for experimental and other scientific purposes (European Community 1986); the United States Animal Welfare Act and Health Research Extension Act (see National Research Council 1996); and specific national legislation, e.g. the Animals (Scientific Procedures) Act 1986 in the UK.

In recent years, attention has been focused on the need to recognize and control the adverse effects of scientific procedures on animals, and similarly on the need to improve and enrich the environment in which laboratory animals spend their lives. There is, however, still a great deal of scope for improving current laboratory practice. Such improvements not only benefit animal welfare, but can also enhance the quality of scientific research, since suffering and distress in animals can result in physiological changes which are likely to increase variability in experimental data and, at worst, may even invalidate the research.

Significant improvements in animal husbandry and laboratory techniques can be made immediately in several ways, but in order to do this, unequivocal and up-to-date information must be readily available. The need to provide such information led the British Veterinary Association Animal Welfare Foundation (BVAAWF), the Fund for the Replacement of Animals in Medical Experiments (FRAME), the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and the Universities Federation for Animal Welfare (UFAW) to establish a Joint Working Group on Refinement. The aim was to set up a series of working parties to define ways that husbandry or procedures could be refined to minimize any distress to, and improve the welfare of, laboratory animals. The members of each Working Party are drawn from industry, academia and animal welfare organizations. Each addresses a specific topic, the proceedings being published in *Laboratory Animals*.

The Working Party recognizes the approach of the current Workshop has been primarily from a UK perspective, and that the peer-reviewed literature in this area is relatively sparse. Nevertheless, we hope that all these reports will be widely circulated in an international forum and that the recommendations will set challenging standards to advance animal welfare and be adopted as current 'Best Laboratory Practice'.

It should be noted that some of the contributors are opposed to the use of animals in experiments that cause pain, suffering or distress. However, they share with many in science the common aim of reducing animal suffering wherever it occurs. The reports of these refinement workshops are intended to help achieve that aim, particularly if they are read in conjunction with other recent reports on the recognition, measurement and alleviation of pain or distress in animals.

The present report, entitled 'Refining rodent husbandry: the mouse', is the third in the workshop series (The first report, Removal of blood from laboratory mammals and birds, appeared in *Laboratory Animals* (1993) **27**, 1–22. The second report, Refinements in rabbit husbandry, appeared in *Laboratory Animals* (1993) **27**, 301–329). It describes ways in which existing husbandry and care of mice can be improved with the emphasis on providing environments that allow animals to express a wide range of behaviours.

1 Introduction and aims of the report

Laboratory rodents account for the majority of animals used in scientific procedures worldwide. Over 10 million rodents were used in Europe in 1991 (European Community 1994)—the first and only year for which European figures are available. The majority were rats and mice (31% and 68% respectively). In the UK where, accurate statistics are collected annually, 2.3 million (87%) of the total of 2.64 million animals used in 1996 were rodents (Home Office 1997). This included 1 496 390 mice (56% of the total), 684 090 rats (26%), 103 218 guineapigs (4%), 9898 hamsters (0.4%) and 7341 gerbils (0.3%). There were also 3546 'other rodents' (0.13%). Improvements in rodent welfare through refining husbandry and procedures would clearly have a profound impact on laboratory animal welfare in general. Better husbandry and care should also result in better quality animals which may subsequently be needed in smaller numbers, so effecting reduction through refinement. This principle is already being applied to other species such as dogs, primates and rabbits (Morton *et al.* 1993) and it seems appropriate to do the same for rodents.

In general, laboratory caging for rodents provides a confined and barren environment. Since the animals spend the greatest proportion of their lives in their home cage, improving or enriching this environment affords a significant opportunity to improve their overall well-being. However, in order to do this properly, the animals' behavioural, and physiological needs must be understood, otherwise alterations to existing housing and husbandry may just reflect human preferences without actually benefitting the animals.

There have been many detailed studies of rodent behaviour (see review by Brain *et al.* 1989) but the significance of the results in relation to laboratory animal welfare is not always clear. Consequently, there is no absolute definition of these animals' needs or how best to satisfy them. The information in current national and international legislation and guidelines on husbandry and care (e.g. European Community 1986, Council of Europe 1986, UK Home Office 1989 & 1995, National Research Council 1996) is limited and ideas have evolved since most of these were published.

This report specifically deals with the husbandry of mice as they are the most commonly used rodent—future publications will cover other species. It reviews husbandry systems in the light of knowledge available in the published literature and of the professional experience of group members. The report recommends ways of improving husbandry and sets out ideal goals, giving the animals the benefit of the doubt where there is no objective definition of their needs. However, the authors recognized that provision of an ideal environment may not always be possible so recommendations are also made for achieving the best possible care (i.e. best practice) in the laboratory situation.

2 The relationship between husbandry and purpose of procedure

Analysis of categories of procedures on mice in UK laboratories (see Fig 1) shows that mice are extensively used in research and testing in human medicine and dentistry, in fundamental research, and in transgenic work. Further breakdown of types of use show that toxicity tests account for around 19% of total procedures on mice. Mice are used extensively in cancer research, accounting for 94% of procedures in this category, together with 68% of procedures in immunology; and 51% of procedures in pharmaceutical research and development. This type of breakdown is likely to be similar in other countries, although figures for individual rodent species are not available.

All mice should be able to benefit from some sort of environmental enrichment even

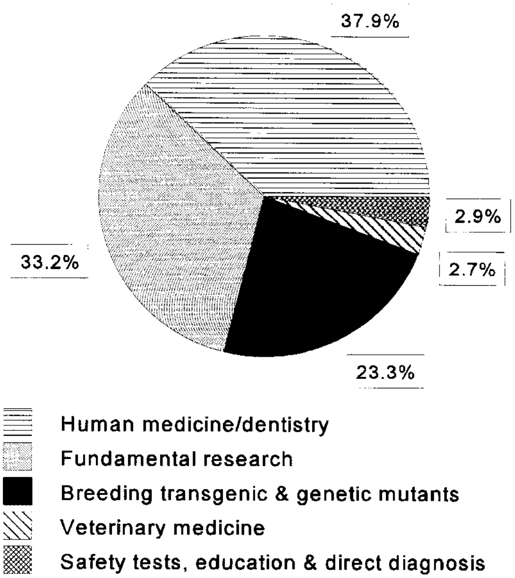

Fig 1 Procedures on mice, 1996

in toxicity testing, where regulatory requirements may be interpreted as restricting the possibilities. The latter view should always be challenged since some sort of environmental enrichment is rarely precluded, provided it is well chosen and documented appropriately.

3 The natural history and behaviour of mice in relation to their husbandry

3.1 The mouse in the wild

The laboratory mouse is derived from a highly opportunistic and adaptable group of species and sub-species which are found, frequently in association with humans, across the world in environments as diverse as cold stores, warehouses, hayricks, Pacific atolls and islands close to Antarctica (Brain & Parmigiani 1990). Some populations seem to be largely surface-dwelling, others inhabit complex burrow systems, depending on the availability of cover and suitable burrowing substrate in different habitats. During domestication mice have been selected for particular characteristics but have, nevertheless, retained many of the attributes of their wild counterparts. Understanding these can provide indicators of the animals' needs (Brain 1992).

Like other small mammals, vulnerability to a wide range of predators has been a major selective force in shaping the behaviour and life history strategies of mice. Thus they are largely nocturnal and have a strong inclination to stay close to safe cover (burrows, vegetation or within the structure of buildings), especially during the daytime or when cautiously exploring unfamiliar territory. Their short life expectancy favours animals that reproduce and develop rapidly, and invest in a large number of small offspring to maximize the chances that some will survive long enough to reproduce (Daly & Wilson 1978, Millar & Zammuto 1983, Read & Harvey 1989, Hurst in press). Such characteristics have had important consequences for the evolution of their senses and communication.

3.2 Senses and communication

Mice are much more dependent on odour cues, audible and ultrasonic emissions, and tactile input than on vision.

3.2.1 Olfaction

Odours are important for communication (e.g. Berry 1981). Keeping mice in cages and subjecting them to procedures that disrupt their odour communication is therefore an important concern.

Mice create patterns of urine deposition that are used in territorial marking and individual and group recognition. In addition, they have a number of glands that produce odoriferous substances which are important in controlling sexual and aggressive behaviours and can also have a potent effect on reproductive physiology. Odours from adult males or from pregnant or lactating females can speed or retard sexual maturation in juvenile females and synchronize reproductive cycles in mature females. Odours of unfamiliar male mice may terminate pregnancies (Brown 1985a, Brown 1985b).

Recent data suggest that strange odours (e.g. associated with humans) can produce stress responses in laboratory mice (Dhanjal 1991). This should be taken into account when cleaning cages and handling animals.

3.2.2 Hearing

Mice have a broad range of auditory sensitivity. They can detect sounds from 80 Hz up to 100 kHz, but are most sensitive in the 15 kHz to 20 kHz range and around 50 kHz. The level of sensitivity varies with age and between strains. Auditory responses have been obtained from mice of 11 days of age. Audiogenic seizures are characteristic of some strains (Gamble 1982) and exposure of such animals to loud sounds at an early age can enhance this sensitivity.

Both audible sound and ultrasound are important. Ultrasound is used in sexual encounters and audible sound is used in agonistic encounters. It is also well established that the ultrasonic 'distress calls' of infant mice elicit recovery responses by their mothers and are good indicators of anxiety (Livesley 1991).

3.2.3 Touch

Touch is an important sense—in times of stress mice retain tactile contact with surfaces (e.g. Berry 1981). Loss of tactile contact with conspecifics seems the most important factor determining the isolation-induced increase in aggressiveness in male mice (Brain & Benton 1983).

The whiskers (tactile vibrissae) are also important in spatial location.

3.2.4 Vision

Mice are adapted to living in low levels of light and vision appears to be a less critical sense for normal behaviour than in other laboratory animals (Strasser & Dixon 1986). Movement detection, as in the startle response, is important, and both visual and olfactory cues have been shown to affect recognition and aggressive defence of the home area (Jones & Nowell 1973).

Mice are insensitive to red light so this is useful for observation purposes. Strong white lights induce and exacerbate retinal atrophy and should therefore be avoided.

3.2.5 Capacity to experience pain, suffering and distress

There is a tendency when dealing with large numbers of similar looking animals to overlook their individuality and capacity to experience pain, distress or suffering. This tendency is even more marked when the animals are small—a sort of 'sizeism'. However, there is no scientific reason to suggest that laboratory mice are any less capable of experiencing pain, distress and suffering than other vertebrates. Indeed, the central and peripheral nervous systems of mice share so many anatomical and functional characteristics with those of humans that they are used as models in the development of analgesics, antidepressants and anxiolytics. In addition, it should be remembered that non-human animals have needs or motivations which are different from our own experiences, e.g. mice are highly motivated to perform nest-building behaviour. Preventing them from carrying out these behaviours can lead to frustration and perhaps mental suffering.

A guiding principle when considering the capacity of mice to suffer and factors which may compromise their welfare is to always give the animal the benefit of the doubt.

3.3 Behaviour of laboratory mice

The behaviours of laboratory mice are quite complex. Detailed ethological analyses reveal more than 40 individual activities and postures that they commonly utilize within their cages (Brain *et al.* 1989). Some of the most common behaviours are:

Maintenance behaviours: e.g. body care, foraging/feeding, drinking, nesting, sleeping.

Non-social investigative/exploratory behaviours: e.g. digging, gnawing, investigation (visual, olfactory, acoustic, tactile), territorial scent-marking, climbing.

Social interactions: e.g. distant and close investigation (visual, olfactory, acoustic, tactile), allogrooming, huddling together/immobile, scent marking for communication, aggressive (threat, attack), defensive (avoid, flee, submit), sexual, parental care, play (in juveniles).

There are over 200 commonly-used inbred and outbred strains of laboratory mice, together with the more recently developed transgenic strains. Individual strains show great variability in behaviour and in the percentage of time they allocate to different behaviours. One example of strain-dependent behaviour is that, in some strains, female mice will attack female counterparts, and some females will even show vigorous nest defence against stronger males. It is recognized that adult males are aggressive to each other, but this behaviour may not be expected in females. Group housing in this situation would require special care. Some strains have specific associated problems, for example nesting behaviour may be very rudimentary, and this can cause problems in breeding and rearing young (Southwick & Clark 1966, Southwick & Clark 1968, Henderson 1970, Brain *et al.* 1982, Guillot *et al.* 1994). The stage of the light–dark cycle, age, sex and social status also have profound effects on behavioural patterns. Some behaviours are also situation- or even supplier-dependent.

Certain behaviours may be indicative of problems with a particular husbandry regime, e.g. injurious fighting behaviour (threat and attack), over-grooming, excessive fearfulness (evidenced variously by avoid/flee, immobility and startle responses), persistent attempts to escape, and a variety of repetitive movements. Stereotypic behaviour, e.g. excessive circling or jumping (Tuli 1993), is also indicative of problems. Such behaviour may occur more frequently during the dark period and therefore is less likely to be detected under conventional lighting regimes.

To define standards of normal behaviour and determine the most appropriate husbandry it is important to find out as much as possible about the stock or strain of mouse to be kept and used. Discussion with the breeder and the animal technicians on the availability, behavioural characteristics and the needs of the animals is essential. Scientists should also seriously consider using genetically-defined strains of mice with a known phenotype that is most appropriate for their scientific purpose, rather than using outbred 'white mice'. This will reduce experimental variance such that fewer animals are needed.

Recommendations:

- **It is very important to understand the normal behaviour of mice and to be able to recognize abnormal behaviour.**
- **It is essential to find out as much as possible about the stock or strain of mouse to be kept and used, both to determine the most appropriate husbandry methods and to set standards regarding normal behaviour, so that problems can be identified as early as possible. Discussion with the breeder and the animal technicians on the availability, behavioural characteristics and the needs of the animals is essential.**
- **Think very carefully about which strain you need and why. It is important to consider the behaviour as well as the scientific suitability of the strain when choosing the most appropriate animal for any given purpose. Wherever possible choose the most docile and easiest to handle. Use genetically-defined strains wherever possible.**

4 Husbandry

It is difficult to specify optimal husbandry conditions for mice in general because of strain differences. Furthermore, the characteristics of some outbred stocks and inbred strains, including some transgenic strains, renders their husbandry and care more difficult. For example, the nude, hairless mutations will need special care to ensure appropriate temperature control and comfort; the males of the SJL inbred strain are very aggressive and cannot be group housed (Crispens 1973). The animals' requirements will also depend on the particular situation—whether they are breeding animals, post-weaning stock or animals undergoing procedures.

When considering the husbandry needs of any laboratory animal it is useful to apply the principle of the 'five freedoms' developed for farm animals by the UK Farm Animal Welfare Council (FAWC 1992 & 1993). These five freedoms include: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury or disease; freedom from fear and distress. The fifth freedom is to 'express normal behaviour'. When applied to mice this means that cages should ideally satisfy the basic physiological and ethological needs of resting, grooming, exploring, hiding, searching for food, gnawing, social interaction, nesting, digging and going into retreats. In short, animals should have some degree of control over their environment.

Current caging falls seriously short of this ideal—the most frequently used cages are of 'shoe box' design and are rather featureless; their size constrains opportunities for activities or enrichment. In the following sections of this report this type of caging is assessed in relation to the needs of the animals, and recommendations offered to encourage better utilization of available systems, together with ideas to improve other aspects of husbandry and care. Ideally, commercially available cages should be redesigned to suit the behavioural and spatial needs of mice, although this is a long-term goal.

A second guiding principle is that it is beneficial to implement husbandry which allows animals to perform the widest possible range of normal behaviour.

4.1 Caging

4.1.1 Cage materials

Cages are made of metal or clear or opaque plastic, frequently with stainless steel wire tops. There is no definitive answer to which type of cage is best for the animal although there is some evidence that opaque cages are preferred (Baumans *et al*. 1987). Different types of material have advantages and disadvantages as follows:

Metal (stainless steel or aluminium)

Advantages	Easier to customize into different shapes to create structural enrichment; durable, long life; easily autoclaved at high temperatures.
Disadvantages	Cold, noisy and textureless—would therefore seem to provide an uncomfortable environment for animals. Stainless steel cages are expensive and heavy. Solid-topped metal cages prevent animals from using the lid for climbing so removing a dimension from the cage; airflow may be restricted.

Plastic

Advantages	Plastic is versatile for moulding although this potential has not been explored. Warmer to touch; lighter to handle; may be more comfortable (O'Donoghue 1994). Clear plastic cages make it easier for staff to observe the animals. The animals may be able to detect staff movement and so would become more quickly accustomed to disturbance in the room.
Disadvantages	Transparent cages are more prone to over-illumination and it is difficult for the animals to get away from the light. Transparent cages reduces the ability of the mouse to hide (a behaviour which seems very important). It is not certain whether mice can see animals in adjacent cages or whether this has any effect on their behaviour.

Some of the disadvantages of the different types of cage can be overcome by ensuring animals have bedding and by adding other forms of environmental enrichment. Use of red filters, or incorporation of an appropriate non-toxic red dye into clear plastic cages (Hubrecht, personal communication), may improve observation whilst reducing the level of disturbance.

Recommendations:

- **Plastic cages appear to have most advantages and are recommended for this reason.**
- **Use environmental enrichment to overcome the disadvantages of different types of caging, e.g. provide plenty of bedding in metal cages and nesting material and hiding places in transparent cages.**
- **Cages with solid lids should not be used without giving the animals facility for climbing and without regularly checking airflow rates.**

4.1.2 Cage size and design

Minimum cage sizes are listed in various guidelines and Codes of Practice (see Table 1). These were derived from a consensus of the best information available at the time they were written, and were based on 'best guesses' rather than any scientifically defined needs of the animals.

The amount of space required will depend on many factors—namely the strain, group size, age, reproductive status, familiarity, time of day and activities being performed. For example, mice group together when

Table 1 Minimum floor space (cm^2/mouse) recommended in existing guidelines

Singly-housed stock mice

Weight (g)	HO[1]	HO[2]	RS/UFAW	ED
< 20	-	200	-	< 60
21–25	-	200	-	60–70
26–30	-	200	-	70–80
< 30	200	-	200	< 80
> 30	200	200	200	> 80
Minimum cage size	200	200	-	-

Group-housed stock mice

Weight (g)	HO[1]	HO[2]	RS/UFAW	ILAR*	Weight (g)	EC
< 20	-	30	-	39–51	10	40
21–25	-	45	-	77	20	60
26–30	-	60	-	95	30	80
< 30	60	-	60	95		
> 30	100	100	100	95	40	100
Minimum cage size	200	200	-	-	Minimum cage size	180

Breeding mice

	HO[1]
Monogamous pair (outbred/inbred)/Trio (inbred)	300 (Plus 180 cm^2 for each additional female plus litter)

Note: The recommended minimum cage height is 12 cm
*The weight ranges have been adjusted to fit in with this table
HO[1]: Home Office Code of Practice for the housing and care of animals used in scientific procedures (1987)
HO[2]: Home Office Code of Practice for the housing and care of animals in designated breeding and supplying establishments (1995)
ED: EC Directive 86/609 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific procedures, Annex II (1986)
RS/UFAW: Royal Society/Universities Federation for Animal Welfare Guidelines on the Care of Laboratory Animals and their Use for Scientific Purposes: I—Housing and Care (1987)
EC: EC Convention ETS/123 for the protection of vertebrate animals used for experiemental and other scientific purposes, Appendix A (1986)
ILAR: Institute of Laboratory Animal Resources, USA. Guide for the Care and Use of Laboratory Animals (1996)

sleeping so at certain times of the day they do not utilize all the space available. This does not necessarily indicate their space requirements at other times of the day when performing different behaviours.

In welfare terms, the amount of space is of paramount importance since it dictates the size of social group possible, the ability of the animals to perform behaviours requiring locomotion or to explore, and the capacity to provide environmental enrichment. (Note that young animals may need more generous space to facilitate the play behaviours that affect their development. Therefore, criteria for determining space requirements should not just relate to floor area per body weight of animal). It has been shown that, when space additional to that of standard caging is provided, mice are highly motivated to enter it (Sherwin & Nicol 1996a) and that they will continue to explore all the available space. This is independent of the amount of additional space gained, suggesting that mice are highly motivated to explore novel areas or that they simply wish to escape the confines of conventional cages.

The volume of space is important, as well as floor area, because behaviours can occur in three dimensions—mice readily climb up and down vertical surfaces (e.g. wire mesh) if these provide sufficient holds. Greater volume also allows provision of additional enrichment. It can be increased by increasing the height of the cage and structuring the environment by providing surfaces (e.g. shelves), climbing facilities, or furniture to enable the animals to utilize the space. However, height should not be unduly increased without providing access to the cage lid, since mice frequently hang from the bars and this behaviour would be thwarted. One low-cost option for routinely providing more space for mice is to house them in large (vacant!) rat cages. The lids need to fit properly to guard against escape. The mesh size should be checked to ensure there are no gaps large enough for mice to push their heads through as they may become trapped.

In the absence of a recognized optimum size, the important point is the *interaction* between the space, the structure of the cage, the animals, and the enrichments provided. Thus, both the quality and quantity of space should be considered. For example, welfare might be improved only marginally, or even be compromised, if mice are provided with increasing amounts of empty space since this may stimulate territorial aggression among males. Cage size should therefore not be increased without increasing cage complexity.

Recommendations:

- **Consider cage sizes given in current guidelines as minimal and provide additional space wherever possible. The aim should be to provide cages large enough to allow inclusion of enrichment so that animals can perform a wide range of different behaviours.**
- **Cage sizes should be considered in three-dimensional terms, i.e. floor area and volume, and in terms of quality as well as quantity of space.**
- **Floor area per body weight per animal should not be the sole criterion for determining space requirements as young animals may need more room for play.**
- **Increased height, and hence volume, is useful but not without provision of access to the lid of the cage and without providing structures to allow the extra volume to be utilized.**
- **Research to determine an optimum practical cage size is required.**

4.1.3 Cage floors

The cages in common use have either solid or grid (mesh) floors. The latter are commonly used in toxicology studies (Hubrecht 1995). They enable faeces and urine output to be observed, and prevent animals from being in contact with, and ingesting, bedding material and minimize coprophagia. (Mice will eat faeces directly from the anus so the latter is ineffective.)

Solid floors allow for the provision of a substrate, grid floors do not. The latter facilitate cleaning but they do not enable animals to carry out many of their normal activities and may cause health problems, for example pressure sores in susceptible animals (Hubrecht 1995) and urological problems in male mice (Everitt *et al.* 1988). Mice seem to prefer a solid resting area and when given a choice they spend more time on solid than grid floors (Blom *et al.* 1996). An important finding is that mice avoided defecating and urinating on the preferred floor—when offered a choice between a solid-floored bedded cage area and an area with a wire mesh base, they deposited urine and faeces on the mesh (Blom 1993).

For these reasons the actual need to use grid floors should always be questioned and the possibility of providing at least some area of solid floor examined. Partial solid/grid floors, however, need careful design if they are to accommodate a substrate.

Recommendations:

- **Solid floors should be used rather than grid floors. The latter should not be used unless there is a good scientific reason, and then only for the minimum period consistent with the scientific objectives.**
- **Mice should always be provided with a solid-floored area for resting/sleeping.**

4.1.4 Bedding and nesting material

Provision of bedding is important in welfare terms for several reasons. It satisfies a variety of behavioural needs—all laboratory rodents spend considerable time manipulating bedding and nesting material, creating tunnels or burrow systems if the depth and consistency is sufficient—and it allows the individual to determine its own micro-environment. It can thus considerably increase cage utilization and is an easy, economical and highly beneficial form of environmental enrichment.

Provision of nesting material is important because mice are strongly motivated to use it (Van de Weerd *et al.* 1998a) and build nests whenever possible, not only during breeding activities, but also to regulate temperature and light levels (i.e. to provide shade). It is therefore essential to provide nesting material in transparent cages. Nest building also enables mice to hide and retreat from conspecifics (Brain & Rajendram 1986, Van de Weerd *et al.* 1997).

Bedding material Good bedding materials include: wood chips, cellulose-based chips, and shredded filter paper. Fine sawdust is not suitable; it cannot be manipulated and particles can cause preputial, eye and respiratory problems. Mice prefer loose materials (Sherwin & Nicol 1996b)—preference tests have shown that when given a choice between combinations of a substrate of wood chips, shredded filter paper or sawdust on wire mesh and solid floors, they preferred the paper substrate.

Materials of larger particle size and shredded or shreddable paper are more appropriate for use on partial grid floors. There is also evidence that mice prefer such materials as they can be used to create nests as well as for burrowing (Rajendram 1984, Blom 1993). Their preferences may also be influenced by the different intensities of sound, including ultrasound, produced by different materials when they scrabble in them (Blom *et al.* 1996).

Over-provision of some kinds of materials, together with the digging activities of the mice, can result in the bedding building up under the water bottle or automatic drinking valve and this can flood the cage. The advantages of bedding clearly outweigh this disadvantage and careful monitoring will in any case minimize the problem. The aim should be to maximize use of bedding materials without causing flooding.

Nesting material Materials that can be used specifically for nest building are hay, straw, clean shredded paper, paper tissues and wood chips. Paper strips have also been used for this purpose with great success. Paper towels or tray papers can be left on the cage top and the mice will quickly pull the sheet through the bars, chew it into pieces and use it for nest building. It has been shown that mice will operate a lever to obtain such material (Roper 1973).

Nesting material preferences and behavioural responses to the material provided differ between strains (Van de Weerd *et al.* 1997).

Only certain types of material will be suitable for newborn young and hence lactating animals. In breeding cages it is important to avoid materials like cotton wool, wood wool or shredded paper that might become entangled around the legs, tails or bodies of neonates and cause injury (Rowan & Michaels 1980). Material which readily absorbs moisture should be avoided. It can stick to the pups, absorbing fluids from their skin surface, causing dehydration and death.

Recommendations:

- **Bedding material is essential for all mice.**
- **Bedding should be provided in sufficient quantities to allow the animals to manipulate their environment and microclimate. A thin layer of substrate as a base is *not* adequate on its own.**
- **Bedding and nesting material provide an easy, economical and highly beneficial form of environmental enrichment which considerably increases cage utilization. Good materials are of large particle size and include wood chippings, shredded paper or paper tissues. Provision of paper for shredding also provides animals with something extra to do.**
- **Nest building material for breeding mice should be such that pups do not become entangled in it. For example, cotton wool is *not* suitable. Do not use highly absorbent materials.**

- **Bedding and nesting material should be uncontaminated and non-toxic.**

4.1.5 Cage additions

There is considerable scope for providing additions (e.g. tubes, shelters) within cages which will encourage activity within the central areas, and increase the opportunity for exercise. The inclusion of different sorts of additions enables different areas to be used for different behaviours so increasing the range of behaviours that can be expressed. It has also been demonstrated that mice reared in an enrichment environment (with wheels, tunnels and toys) possess more hippocampal neurons than litter mates reared in standard cages with no additions. It is likely that these extra neurons contribute to the enhanced performance in spatial learning tasks displayed by the 'enriched' mice compared to the controls (Kempermann *et al.* 1997). It is important to monitor additions to ensure that they do not cause any problems and to evaluate their benefit.

Baffles and barriers Mice attempt to divide their cages into separate areas for defaecation, feeding, resting, urination and food storage. Although these divisions may be based on odour marks rather than physical divisions, provision of partial barriers within the cage may be helpful to facilitate this behaviour. It increases the complexity of the environment and might also increase the size of the cage as perceived by the mice.

Vertical partitions appear to reduce fearful or anxious behaviour in a novel environment in some strains of mice (Boyd & Love 1995). They may be superior to horizontal partitions—perhaps they represent burrows or satisfy light-related wall-seeking behaviour. Both opaque and transparent partitions have been used to similar effect (Chamove 1989). There are a number available commercially.

Shelters and retreats Mice will use structures such as tubes, cans and empty water bottles for hiding or sleeping in, and in some strains, as latrines. Cardboard tubes are particularly versatile in that they provide opportunities for climbing, chewing and manipulation. There is also some evidence that mice seek refuge in tubes or other retreats during attempted capture or on being startled. Short tubes can therefore be useful for subject removal.

The structure of any insert is important since, for example, inserts with only a few openings might increase aggression among male mice (e.g. Haemisch *et al.* 1994).

The effect of different materials or design varies between strains. For example, some strains of mice will distinguish between nest boxes made of different materials (Van de Weerd *et al.* 1998b, in press) or differing in shape (Buhot–Averseng 1981), while other strains do not indicate a preference for particular designs of shelter or tube or for opaque or transparent tubes (Sherwin 1996).

Carefully designed cage additions can be used in toxicity studies—an opaque or red (i.e. opaque to the mouse) plastic shelter would provide an easily cleanable environmental enrichment item. Enrichment is even possible in metabolism cages. McSherry (1997) demonstrated that a variety of cage furniture including a shelf, box and pipe could be used in metabolism cages without affecting urine collection.

All cage additions need to be non-toxic, easy to clean or disposable.

Recommendations:

- **Provision of additions such as baffles, barriers, shelters and retreats within cages is recommended since this increases the range of behaviours by allowing different areas to be used for different behaviours.**
- **The structure of cage additions should not be such as to increase aggression. Enough shelters or retreats, or openings to single structures, should be provided to prevent any associated aggressive behaviour.**
- **All additions need to be non-toxic and easy to clean or disposable.**
- **All additions should be monitored in use to ensure there are no harmful effects to the animals.**

4.2 Nutritional enrichment

Food variety can be used to enrich the lives of mice by providing different tastes and textures. In addition, different foods are handled in different ways which can increase the diversity of behaviour.

Expanded diets are generally considered to be more palatable than pelleted diets, possibly due to the differences in texture and flavour. Seeds, fresh vegetables, fruit, and bread all provide variety and can be incorporated in small quantities. However, the effect of any variations in diet must be monitored to ensure there are no adverse effects to the animals (such as failure to gain weight or nutritional problems) or to the science. For example, the results of behavioural experiments could be modified because diet can affect odour cues (Brown & Schellinck 1992).

Getting mice to 'work' for food may also have an enriching aspect. Furthermore, in nature, seeking food is one reason for animals to exercise—the ease of obtaining food in cages can consequently inhibit general activity. This, combined with *ad libitum* feeding means that many laboratory mice suffer from obesity with a consequential decline in life span.

Recommendations:

- **Consider providing variations to the standard laboratory diet to incorporate foods with different textures and flavours, but monitor animals for adverse effects.**
- **Monitor animals for signs of obesity with any diet and reduce food intake if this occurs.**

4.3 Establishing social groups

Mice are social animals and, where possible, should be maintained in stable and harmonious social groups. There is generally no problem in doing this with young animals and non-breeding females. Aggression may occur but provided that the groups are carefully set up, with suitable individuals which remain together as a group, any conflict is generally 'ritualized' with animals avoiding injury. Housing males together is more of a problem, particularly with small groups of two or three (Evans & Brain 1975). Nevertheless, it is important to achieve successful group housing of males since more use could then be made of them and wastage due to the use of only one sex would be reduced.

The amount of conflict that occurs is highly strain dependent but other factors including the age of the animals, the experiences of the individual, the group size, cage size and the situation, also have a critical influence (Brain & Parmigiani 1990). Conflict may be reduced by the provision of carefully designed environmental enrichment. Close observation of the animals' behaviour is essential.

Group formation The formation of a social group is not simply a matter of adding individuals together. Each animal plays a role in the group, often dictated by its age, sex, position in the hierarchy or reproductive condition. This needs to be considered when selecting animals to form groups and when designing cages to accommodate them.

It is a good general principle to start with weanlings that know each other; if possible obtain them pre-grouped at weaning from the breeder. Single sex groups are best set up prior to puberty since levels of aggression can escalate at this time especially between unfamiliar males (Barnard *et al.* 1991). Groups should be established in clean cages as home cage odour cues induce residents to attack intruders in their territory, while the substrate odours of unfamiliar males can also induce aggression (Brown 1985a). Always monitor animals immediately after grouping and when regrouped after cleaning.

The maximum group size is partly determined by the cage size and the project design as well as by the age of the animals. In very small groups (especially two males) there may be an excessive amount of stress on the subordinate(s). Group sizes and constitution should be kept consistent; hormonal measurements confirm that altering these is more stressful than keeping them constant. It is poor practice to set up established groups and then keep moving the animals around.

Mice do not adapt well to repeated changes of social partners and post-breeding mice (especially males) should not generally be reintroduced to same-sex cage mates (Brain & Bowden 1978).

Recommendations:

- **Mice should be kept in stable harmonious groups wherever possible. The number of**

animals per group depends on factors such as their age, the cage size and the provision of environmental enrichment.
- **Be aware that some strains are more aggressive than others and that many factors can influence the amount of conflict. Seek advice before setting up groups.**
- **Use young animals to establish the group preferably grouped at weaning by the breeder.**
- **Do not move animals around between groups once they are established. Try to keep both group size and composition consistent.**
- **Monitor groups for signs of aggressive behaviour and injury particularly after initial formation and cleaning cages.**

4.4 Cleaning and odour cues

There are two conflicting pressures—the need to clean cages for hygiene and health, and the need not to disturb scent marking patterns too frequently.

With respect to hygiene, some mouse diseases and infections are exacerbated by atmospheres high in carbon dioxide and ammonia. The build-up of ammonia can vary with the strain and age of animals and in different parts of the cage, for example under the food hopper (Eveleigh 1993). Continual exposure to wet bedding is also detrimental to animal health. Cleaning, however, disturbs scent marking patterns and this stresses mice and may even produce transient conflict in group-housed males. This response may also be influenced by strain, sex and environmental factors including lighting, noise and relative humidity.

As a general rule, it is important not to over-clean cages and to make the cleaning process as consistent as possible. For example, do not wear strong scents as unusual odours can stress mice. The frequency of cleaning depends on factors such as the cage size and stocking density, the degree of soiling and levels of ammonia, and whether the animals are breeding, stock or on procedures. One firm rule is that animals should always have dry bedding. As a guide, for practical purposes, a clean-out frequency of once per week for the standard stocking densities in use is generally adequate.

A variety of clean-out procedures are currently used in different laboratories. These include:

(a) put the animals in a new clean cage and give them fresh bedding;
(b) put the animals in a new clean cage with 90% clean and 10% old bedding;
(c) retain the old cage and replace all bedding;
(d) retain the old cage and remove soiled areas.

There is no definitive opinion as to which is the best method and this is clearly an area where more research is needed. It will depend on the individual animals and the situation. In order to decide the best cleaning procedures, it is helpful to understand the interaction between odour cues and social responses. This has been studied by Gray and Hurst (1995) who found that replacing all sawdust but retaining the uncleaned cage base and top (point (c) above) provoked maximum aggression within groups of males. This is almost certainly because it removes the substrate cues used to recognize current group members (Hurst *et al*. 1993) but retains the home cage territory cues. The effect of retaining some of the sawdust ((b) & (d) above), was not tested. However, substrate odour cues are important in maintaining tolerance (Hurst *et al*. 1993) and if the odour of one group member is selectively 'removed' from the shared substrate he is targeted for attack as if he has dispersed from the group. The observation that retaining some bedding reduces aggression is therefore to be expected.

The feeling of the Working Group was that strategy (a) above can be used for all stock and is the best method for aggressive males. Strategies (b), (c) and (d) are all appropriate for breeding animals. It is especially important not to disturb females and their litters too much as this may result in mismothering or cannibalism, and because neonates are especially susceptible to stress. Therefore, avoid cleaning cages in the first week of the new litter. Whatever method is used, it is important to monitor animals after cage cleaning for increased stress, e.g. fighting behaviour.

Recommendations:

- **Animals should always have dry bedding.**
- **Do not over-clean cages and make husbandry procedures as consistent as possible.**
- **Always monitor animals for increased aggressiveness after cage cleaning.**
- **Minimize disturbance to breeding females and their litters.**

4.5 Housing rats and mice in the same room

As a general principle, keeping mice in the same room as rats is not recommended because the latter are their natural predators (Draghi & Brain 1993). There may be exceptions where animals have become habituated to each other, e.g. in some breeders' colonies.

4.6 The laboratory environment: lighting, temperature, humidity, noise

Details of approved conditions are given in the various publications, e.g. Home Office Codes of Practice (Poole 1987, Home Office 1989 & 1995, National Research Council 1996). Lighting and noise are particularly important in the case of mice and therefore further details are included here. Some strains have specific requirements (e.g. nude and ob-ob mice may have problems with thermoregulation) and it is important to be aware of these.

4.6.1 Lighting

Lighting is important both because it affects activity cycles—the importance of light to dark cycles in regulating circadian rhythms and stimulating and synchronizing breeding cycles is well documented (Clough 1982)—and because it can cause retinal damage particularly in albino animals. A daily cycle of 12 : 12 is usual. Since mice are most active at night, consideration should be given to having the dark period during the working day, starting at 15:00 h or 16:00 h, and using red light (e.g. as provided by 40 or 60 W red bulbs or red fluorescent light strips) for monitoring.

Light levels within the cages are more important than the light level in the room. The distribution of cages in the racks and the room itself therefore needs to be considered since the top racks will be exposed to quite intense light and associated heat. This can be avoided by fitting baffles, e.g. a metal sheet, or shaded tops over the top cages. Animals should be given the opportunity to withdraw to shaded areas within the cage, for example by provision of adequate bedding/nesting material. This is especially important for breeding animals and for those housed in transparent cages.

More use should be made of subdued lighting for monitoring purposes. However, it is also important to ensure an adequate minimum light level for appropriate routine husbandry practices, clinical inspections and for staff safety.

Recommendations:

- **Keep light levels low in general, but allow enough light for routine husbandry, inspection and safety. Use red light for night-time inspection.**
- **Protect the top rows of cages from excess light.**
- **Provide shaded areas and nesting material in cages.**

4.6.2 Noise

Mice have a broad range of auditory sensitivity and loud sound can affect them adversely during their development and throughout their lives. Juvenile animals can become sensitized to loud sound including ultrasound, which can increase the incidence of convulsive behaviour in response to sound, later in life. Some strains of mice (e.g. DBA/2) are especially sensitive in this respect (Gamble 1982). The noise levels in some animal units can therefore cause stress and may interfere with communication with conspecifics, e.g. between mothers and offspring.

High levels of sound can be produced during routine maintenance. Ultrasound is produced by cleaning devices, pressure hoses or running taps (Sales *et al.* 1988) and computer monitors. The latter are commonly used in the presence of mice but it is preferable to put them outside the animals' room or screen them, e.g. with a thick piece of polystyrene or foam rubber.

Alarm systems, telephones and door bells within rodent facilities should be designed to operate at frequencies less audible to the rodent ear, e.g. below 500 Hz. No upper limits for levels of ultrasound have yet been identified, but it is preferable to keep levels as low as possible. The incidence of ultrasound can easily be assessed using a commercially available bat detector covering frequencies between 20 and 100 kHz (Pye 1983).

It has been suggested that a constant background noise (e.g. radio music) has benefits in making animals less jumpy and easier to handle although there appears to be no scientific evidence for this. Music provides background disturbance of a mild nature, and may thus minimize the disturbing effects of sudden noises. It could, however, stress some animals and in any case should not be too loud. It is possible that radios have more enrichment value for humans than animals, but if this leads to more satisfied personnel it is also likely to have beneficial consequences for the animals.

Recommendations:

- **Keep noise, both audible and ultrasound, within the animal unit to a minimum and avoid sudden loud disturbances. Radios should not be too loud.**
- **Equipment producing ultrasound (e.g. computers) should be well screened, or preferably used outside the animal rooms.**

5 Health and quarantine

The aetiology, effects and control of diseases of the laboratory mouse are the subject of numerous standard texts, e.g. Foster *et al.* (1982), Poole (1987), Laber-Laird *et al.* (1996) and National Research Council (1996), from where specific detailed information can be obtained.

Husbandry should aim to maintain animals in the highest standards of health, to minimize incidents of overt clinical disease, zoonotic risk to humans and interference with experimental results. It is important to obtain animals of high health status and to prevent compromise of their health within the animal facility. This involves good hygienic practices. A period of quarantine is recommended for any incoming animals which may pose a risk to existing stocks.

Since disease affects health and welfare, it is recommended that regular health surveillance is carried out involving, for example, clinical examination and microbiological monitoring, as deemed appropriate in consultation with the establishment veterinarian. Recommended health monitoring schemes have been published for experimental animals (Federation of European Laboratory Animal Science Associations 1996) and for those used for breeding (Laboratory Animal Breeders Association 1993). An animal's susceptibility to disease can be increased by stress. Stereotypic behaviour, e.g. repetitive circling, bar chewing (see Section 3.3) may be a sign of an inadequate environment. Refinement of husbandry practices may help minimize stress and thus susceptibility to disease. However, care must be taken when providing environmental enrichment, that this will not cause injury or disease to the animals, e.g. from physical damage or by the introduction or transmission of infection.

Recommendations:

- **Obtain animals of high health status.**
- **Regular health surveillance should be carried out involving, for example, clinical examination and microbiological monitoring as deemed appropriate in consultation with the establishment veterinarian.**
- **Care should be taken that anything introduced into the cage cannot injure the animals or introduce infection.**

6 Catching and handling

Information on catching and handling mice is available from a number of sources (Poole 1987, Institute of Animal Technology 1991, The Biological Council 1992).

These are potentially highly stressful procedures (Porter & Festing 1969, Kramer *et al.* 1993) and staff should be trained by experienced handlers who can demonstrate methods that reduce the risk of injury and stress to the animal and the handler.

Catching animals can be facilitated by some environmental enrichment objects, e.g. tubes, but care should be taken that such objects do not make the procedure more difficult. It is necessary to strike a balance between the animals' and the users' needs. Providing a complex environment only to tear it apart chasing the animal around in an attempt to catch it could be very stressful.

Handling must be carried out in a firm, confident and gentle manner, with care being taken to limit restraint to restriction of movement without crushing or squeezing the animals. Transferring animals between cages should be done carefully. Some facilities use rubber-ended forceps or photographic print tongs, picking the animals up by the base of the tail. This may be less threatening than a human hand and can make it easier to separate and catch individuals. When handling experimental animals, prior knowledge of any experimental procedures that the animal may have been subjected to is essential, especially if it impedes the way in which the animal may be lifted, handled or restrained.

There is debate about whether it is more stressful for mice to be handled little, or often. This, in part, depends on the situation. In some circumstances, for example with breeding females or when studying animal behaviour, it may be better to leave the animals alone provided that they can be observed satisfactorily. However, frequent sensitive handling has the advantage that it allows closer observation of animals and any problems can be detected early. It may provide a form of enrichment, especially for single-housed animals who may enjoy a period of socialization. For animals used in procedures, regular handling (at least daily) can also be beneficial in that it conditions animals and may enable subsequent procedures to be undertaken more easily, thereby reducing any associated stress. This in turn can have a beneficial effect on results and reduce wastage.

Recommendations:

- **Handling must be carried out in a confident, firm yet gentle manner and staff should spend time becoming competent in this.**
- **Before handling animals which have undergone procedures, check whether the nature of the procedure has affected the way the animal can be handled so that the potential for additional distress can be avoided.**
- **Judicious use of environmental enrichment objects can make catching easier and less stressful for the animals.**

7 Identification

The size and similarity in appearance of mice means that identification of individuals is difficult and there is currently no non-invasive method of permanently marking them. Before using any procedure always establish whether identification of individuals is really necessary, or whether it is sufficient to identify animals according to the cage of origin. Non-invasive methods are advised for pre-weaned animals.

Non-invasive semi-permanent methods

- Marker pens: one application, e.g. a circular band at varying positions on the tail, can last for up to 3 weeks depending on the extent of grooming and whether the animals are group housed.
- Hair clipping: this will last from 2–6 weeks.
- Hair-dye: provides more long-term identification.

The potential toxicity of marking substances must always be considered.

Permanent methods It is better to use non-invasive methods wherever possible. Note that it is the policy of some journals not to publish results where injurious methods of identification have been used. Permanent marking methods should always be carried out by competently trained staff.

- Microchipping: the most satisfactory means of permanent identification is through subcutaneous electronic implants incorporating a unique identification code detectable by a compatible reader. The implants are very small and, provided the technique is performed competently by experienced staff, cause little obvious distress. Migration of the implants subcutaneously or within the body cavity can occur, and it is therefore important both for welfare reasons and to allow recovery of the implant, that insertion is performed in the correct position, e.g. between the shoulder blades. Subcutaneous implants give no external indication of the animals' identity, which may make them unsuitable for some applications.
- Tattooing the tail: a local anaesthetic spray should be used.
- Ear-notching and punching: care should be taken to use sharp punches which do not tear the tissue.
- Ear-tags: these are small and difficult to read and might be irritating to the mice or catch on the caging.
- Freeze marking with spots of liquid nitrogen: this is useful for marking pigmented strains.

Recommendations:

- **Determine whether marking of individual animals is necessary.**
- **Always use non-invasive methods for neonatal animals, and other animals wherever possible.**
- **Subcutaneous transponder implants provide the most satisfactory method of permanent identification.**
- **Toe amputation should *never* be used.**

8 Balancing supply and demand

An important consideration when designing experiments is the availability and delivery times of the animals required. The size of breeding colonies, and thus wastage rate (i.e. animals culled as surplus to requirement and not sold or subsequently used as breeding stock), could be considerably reduced if a more organized approach were made to both requirements and ordering. This has both welfare and economic advantages.

Mice used in procedures are bred and supplied either in-house or by commercial establishments. The larger commercial colonies are the most efficient because of the scale and economics of their production. Smaller colonies are considerably less efficient and a wastage rate as high as 50% occurs in some establishments. This could be significantly reduced if users improved their project management. It is important to plan procedures and place orders well in advance so that breeders can plan their production to match (perhaps commercial breeders could provide incentives for forward planning). It is unrealistic, for example, to expect the less widely used strains to be available 'off the shelf' at short notice. If 6-week-old mice are required, then 10–12 weeks pre-order time is not unreasonable allowing for organization of breeding, gestation and weaning times. Recovery and quarantine times after the arrival of the animals will also need to be taken into account.

Wastage also occurs where there is a demand for only one sex of animal. The need to use only one sex should always be challenged on a scientific, rather than a 'custom and practice' basis.

Good communication about animal availability within an animal facility will further reduce wastage. If surplus stock have to be killed every effort should be used to utilize them for *in vitro* work or as food for zoo animals to reduce breeding specifically for these other purposes. Some commercial breeders already do this.

Before ordering animals, always make sure there is sufficient space to house them.

Recommendations:

- **Use the supply which is likely to incur least wastage.**
- **Good planning of experiments to predict animal requirements as accurately as possible and in good time, and ordering accordingly is essential. Advanced planning leads to planned production and consequently less wastage.**

- **Question the scientific and practical need to use a single sex.**
- **Aim for a wastage rate of no more than 10%.**
- **Before ordering animals, always make sure there is sufficient space to house them.**

9 Transport

Transport of animals is potentially fraught with problems, whether transportation is external (from one establishment to another) or on site (into and between animal units, buildings, floors, rooms, barrier units, isolators). Guidelines regarding transport to the establishment are provided by the Laboratory Animal Breeders Association and Laboratory Animal Science Association (1993), but there may be little or no guidance on receipt of animals and subsequent on-site transport (Tuli *et al.* 1995). A well defined up-to-date standard operating procedure, clearly displayed and vigorously enforced, is therefore essential. All personnel need to be clear as to their role and what is expected of them. Clear lines of communication are important with both dispatcher and receiver appraised of all relevant information.

9.1 Acclimatization

Transport, even short journeys, can stress an animal and disrupt its physiology (Weisbroth *et al.* 1977, Landi *et al.* 1982, Tuli *et al.* 1995, Van Ruiven *et al.* 1996) so when mice are bought in, or moved between sites or units, adequate time must be allowed for them to recover from any transport stress and acclimatize to a new environment before procedures are carried out. A minimum of 5 days should be allowed for acclimatization once animals arrive on site, longer times may be necessary depending on the needs of the strain and the nature of the journey. Twenty-four hours should be allowed after on-site transport.

The supply of food and water during transport may shorten the adaptation period and is therefore recommended (Weisbroth *et al.* 1977, Van Ruiven *et al.* 1996). Peters and Bywater (1983) give examples of how this can be done.

9.2 Collection and dispatch of animals to or from the site

Road transport vehicles should be appropriate for the purpose, i.e. have air-conditioning and be designated for carrying animals. Parcel vans or private cars must not be used for animal transport. Other general principles are:

- Ensure Laboratory Animal Breeders Association/Laboratory Animal Science Association guidelines are followed.
- Be prepared for problems.
- Ensure all relevant telephone numbers are accessible at all times.
- Ensure appropriate staff will be available at all times.
- Never attempt to collect or deliver rodents from airport quarantine areas without previous experience. Where feasible, pay for experts to arrange for necessary import/export permits and to collect/deliver rodents to/from the airport. Use approved quarantine collection and delivery companies.
- Ensure arrival/departure times are known in advance and contingency plans exist to cope with unforeseen delays.
- Be aware of national regulations and international guidelines (e.g. European Commission 1995, International Air Transport Association Live Animals Regulations 1997) and conventions on the transport of animals and ensure these are applied.

9.3 Receipt of animals

The use of general store areas for receiving animals should be avoided and for preference, a designated temperature controlled area assigned.

Deliveries should be by prior arrangement with the sender and estimated arrival times known. Unacceptable delivery times, e.g. early or late, should be clearly stated since it is unlikely that animals will be re-housed by the sender if deliveries cannot be accepted, and this could result in the wastage of animals. Signed documents should be available for inspection.

Animals should only be received or dispatched by a trained person—i.e. one who is aware of the need to minimize delays and of how to do this with minimum stress to the animals. A maximum wait time from delivery to caging should be agreed. This should not exceed l–2 h. Animals should never be left unattended.

Animals should be examined carefully on arrival and the inside of the box checked for extra animals, dead or sick animals, litters born or aborted during transit. The general health status should be noted and signed for. If there are any problems the local veterinarian and supplier should be promptly informed.

Names of persons available in an emergency and how to contact them must be clearly displayed.

9.4 On-site transport

Problems for animals can arise from: inappropriate containers, noise (e.g. from stiff polythene/plastic bags, metal trolleys and/or containers), vibration, use of lifts, the mechanics of getting them through barriers, sudden changes in environment (temperature, lighting, relative humidity) and microbiological contamination. Staff can be affected by animal allergens and microorganisms.

9.4.1 Containers

The type of containment system is important. It should be escape proof and cause the animals minimum stress. Wherever possible mice should be transported in their home cage, either within a suitable outer container or covered, for example, with a filter cover. Water bottles should be removed until the destination is reached to prevent water dripping and soiling the sawdust. For longer transport periods, e.g. if animals are to be dispatched off site, 'solid' fluid such as agar, fruit or potato, should be provided.

For transport along corridors, animals should be housed in filter boxes or filter cages to protect them from the external environment and to protect staff from allergens. If the boxes or cages are to be placed inside something else, then additional risk and stress factors must be assessed, particularly if animals cannot be seen from the outside. For instance, if cages are placed inside plastic bags for protection, noise produced by the movement of the bags may cause severe distress to the animals. Metal containers used to house boxes or cages may also cause unacceptable stress to animals due to sharp intermittent increases in noise levels.

If there is no alternative other than to place animals in sealed containers, e.g. to pass through a barrier, then this operation must be treated as potentially extremely dangerous for the animals and should be treated accordingly.

When movement involves taking the animals outside, i.e. between buildings—fluctuation in the environment could cause severe distress, especially during winter. Containment systems should seek to ensure constant temperatures. Use a well insulated container in addition to the transport box and plenty of bedding.

9.4.2 Trolleys

Trolleys should be designed to minimize noise—pneumatic wheels are an advantage as is rubber matting to cover shelving. (Note: Carrying boxes by hand is not recommended because of the chance of tripping and dropping boxes.) Where animals are moved regularly, the development of specialized equipment, such as an isolator with pneumatic tyres designed for the purpose is recommended. Any changes to the existing systems should be monitored to ensure that new problems are not created.

9.4.3 Lifts

Transporting animals in lifts can be stressful and an overnight recovery period may be necessary. The noise levels in lifts should be assessed—this includes the type and operation of the doors, the lift motor and movement along the lift shaft. Avoidance of problems may require bypassing floors by using an override key and closing manual doors with the minimum amount of force. Entry to the lift should also be restricted when animals are being transported.

If at all feasible, monitor routes to determine the noise levels (including ultrasound) that may be encountered.

9.4.4 Decontamination procedures

If animals are to be received behind a barrier unit via a pass-through hatch, then the method of decontamination must be agreed and any resultant stress to the animals assessed. This could involve wiping the outside of the box and spraying the chamber with disinfectant. Wait times within the chamber must be agreed and the person on the inside of the barrier must be aware of the full procedure. A balance should be struck between the long-term health of the animals and the short-term stress involved in the procedure.

Recommendations:

- **Ensure Laboratory Animal Breeders Association/Laboratory Animal Science Association guidelines are followed.**
- **Animals must be allowed to acclimatize to a new environment for a minimum of 5 days after arrival on site and before use in procedures.**
- **Ensure there is a well-defined standard operating procedure for on-site transport.**
- **Wherever possible, mice should be transported in their home cage within an appropriate containment system designed to reduce stress, particularly that due to noise or vibration.**
- **Where animals are placed in sealed containers, e.g. to pass through a barrier, this should be treated as a potentially high-risk operation. All containers *must* be clearly labelled and have a clear window for observation.**
- **Keep transport times to a minimum and allow acclimatization for 24 h after on-site transport.**

10 Animals in containment systems

The requirement for more specialized designs in rodent caging, together with more stringent human health and safety requirements and the need to reduce space requirements and hence costs, has led to the design of systems which require the minimum human intervention or intrusion into the cage. This, in turn, has led to a reduction in the close interaction between staff and animals which has always been considered to play such an important part in good husbandry practice.

Examples of caging or systems designed to prevent zoonosis, cross-infection or give protection from allergens or carcinogens include positive and negative pressure isolators, individually-ventilated cages, filter top cages and air filtration cabinets.

10.1 Areas of concern

10.1.1 Reduced interaction between staff and animals

Staff are physically divorced from the animals because cages will often be kept in cabinets; cage lids can only be removed in suitable 'Carrier' cabinets; and often, quite heavy gloves must be worn when handling animals or equipment. In addition, animals may not be given enough attention if doing so requires taking them to a 'station' or resetting the system.

10.1.2 Restricted visibility

It may be difficult to see inside cages without removing them from racks or cabinets. Visibility is further restricted since racks are high (up to 18 feet) and steps may be required to reach the top rows. In such cases there must be a system in place to ensure that animals are inspected regularly.

10.1.3 Space and design

The design and expense of current rack systems limits the space available to increase cage size and the flexibility to change cage design, i.e. these systems are leading to increasing cage standardization.

10.1.4 Environment

An isolator will have its own internal microenvironment and it should not be assumed that this is the same as the external environment surrounding the isolator. Each isolator should therefore be treated as an individual room. It is important to have a system for detecting changes in the microenvironment, although exclusive reliance on automatic monitors is not advisable.

Temperature The temperature within the isolators may be several degrees higher than the surrounding ambient temperature either as a result of the animals' body heat, heat transferred from the motors into the isolator, or poor airflows within the isolator. Maximum and minimum internal temperature recordings should be kept and the external room temperature set to provide the optimum environmental conditions within the isolator. Increasing airflows may affect the internal temperature and cause draughts.

Relative humidity Relative humidity within the isolator should be monitored and recorded (although this will usually reflect the relative humidity surrounding the isolator). Water spillage within the isolator will increase the relative humidity.

Isolator failure All isolators should have two fans, one bringing air in and the other taking air out. This system is preferable to a single motor because the air changes per hour can be increased without changing the pressure, and should one motor fail, air will still be fed into the isolator. Where there is a total system breakdown, an isolator full of mice will generally contain enough air for approximately 6 h before oxygen depletion is seen to affect the animals. It is therefore essential that a continuous 24-h warning system is in operation—either by regular visual checks or alarm monitors. Details of emergency procedures must be displayed and competent staff must be available on a 24-h basis to deal with emergencies. Spare parts for isolators must be readily available.

Lighting levels The design of isolator may affect the lighting levels. Lighting should be monitored and adjustments made in the design, or extra lighting provided, if necessary. If the light levels are too high the animals must be given the opportunity to hide.

Noise There can be considerable noise from motors, fans and general vibration. Old or worn motors and fans should be inspected and, if necessary, should be replaced on a routine basis.

10.1.5 Sterilization

The use of disinfectants or fumigants within the ports of isolators may result in unacceptable levels of chemical contamination within the isolator. Therefore, the minimum amount required should be calculated and the possible effects on the animals investigated. Peracetic acid should not be used except where animals are maintained in a germ-free state. Ten per cent formaldehyde solution should not be used except when the isolator is empty and even then filters should be checked for residual amounts after fumigation. A supply isolator should be used to pre-sterilize goods inwards (or outwards) and lower the stress, and possible risks, to the mice by reducing the number of times the barrier entry port has to be used.

10.1.6 Surgical procedures

To minimize the risk to human or animal health and to minimize stress through movement of animals, the performance of minor procedures up to use of general anaesthesia for 'minor' surgical interventions (e.g. subcutaneous implantation of tumours or minipumps) within the home isolator, is reasonable. However, for 'major' surgery animals should be moved to a surgical isolator or a laminar flow cabinet. Unless disease control or other containment requirements dictate otherwise, isolator reared or maintained animals should be removed from the isolator before humane killing.

10.1.7 Staffing levels

Working within isolators is time consuming, therefore adequate staff must be available to ensure husbandry and technical tasks are carried out correctly.

Recommendations:

- **There are problems common to all containment systems and any system should be examined very carefully before instal-**

lation to ensure animals will not be caused undue stress. Systems must then be regularly monitored during use.

- **Whenever barriers systems are required, scientists and animal care staff should endeavour to maintain similar standards to those which can be achieved in conventional housing systems, i.e. as recommended for housing and care included in this document.**
- **The methods to be employed in examining animals, cleaning cages and carrying out surgical procedures, need to be carefully considered and clearly stated.**
- **Adequate monitoring systems to detect equipment failures must be in place and checked on a regular basis.**

11 Genetically-modified mice

The general principles in this document apply equally to transgenic mice. The production and breeding of such animals, however, is a relatively new science which presents additional unique problems with respect to their husbandry and care. These relate to any expected or unexpected adverse effects of the construct; superovulation procedures in donor animals; mating; foster mothers/recipient animals and breeding. These concerns will be addressed in a future refinement working group report.

Every strain should be considered as a separate entity and its needs critically evaluated and provided for. Careful and continuous observation of each transgenic strain is essential and everything should be done to ensure that a high health standard is maintained.

Recommendations:

- **The general principles in this document should be applied to transgenic animals.**
- **Every strain should be considered as a separate entity and its needs critically evaluated and provided for.**
- **Animals should be maintained by experienced staff, trained to observe animals so ensuring that welfare or health problems can be quickly identified.**

12 Wild mice

Wild mice are considerably more wary of human contact than laboratory strains and whether wild-caught, or bred in captivity, are more difficult to maintain and to work with in the laboratory. Because of the practical difficulties of working with them, their different ethology and the possibility that stress is greater in these 'undomesticated' species, their use should be carefully considered and specific scientific justification made before using them.

12.1 Housing

Wild mice do not readily adapt to confinement and will attempt to escape at every opportunity. An ideal housing system would be a high-walled enclosure (at least 0.8 m taller than the highest jumping point) containing ample covered sites for them to rest, nest or hide in, or alternatively, an enclosed tunnel system. A number of authors (Crowcroft 1966, Reimer & Petras 1967, Lidicker 1976, Poole & Morgan 1976, Van Zegeren 1980) provide useful and different ideas on different scales.

In the laboratory, wild mice can be maintained in cages with tight-fitting lids. They are generally smaller than laboratory strains; adults weigh 11–26 g and can squeeze through surprisingly small spaces, so avoid leaving holes for water bottle spouts unplugged. Do not use mesh 10 mm or wider, as they will often attempt to squeeze through and may get stuck at the neck or waist. Suitable shelters e.g. tubes, nestboxes should always be provided as should bedding and nesting materials (see section 4.1.4).

12.2 Handling

Special consideration must be given to how wild mice are approached and handled to avoid distressing the animals or being bitten. Animals should be maintained under reverse lighting conditions and approached and handled under red light. This is because attempts to catch mice in daylight will usually cause persistent and distressful leaping and scurrying. A slow calm approach is essential.

Even regularly handled laboratory stock are not easily picked up, but all will readily learn to cooperate with handling techniques that allow them to avoid direct contact with humans, for example, by allowing them to run in and out of tubes or small boxes. Nestboxes or tunnels with sliding doors or covers provide a stress-free method for catching and moving mice around and clear plastic or perspex tubes allow close inspection without evident stress, even in daylight.

Cages or handling boxes should always be opened within a high smooth-walled handling bin (minimum height 60 cm) as wild mice appear to be spring-loaded and will rapidly leap out. However, after their initial escape response, they should quickly settle to explore their surroundings. Small shelters or shredded paper within the handling bin will facilitate catching.

12.3 Health

Special precautions should be taken with wild-caught animals. They are likely to carry a number of parasites and diseases which can infect humans as well as other animals, and so should be properly quarantined and treated before being housed in the same unit as laboratory strains. Veterinary advice should always be sought. Mice from some populations can also be very limited in the food that they will accept and can starve to death even when provided with ample laboratory diets. Food intake should be carefully monitored over the first few days in captivity and mice gradually weaned onto laboratory diets if necessary and possible.

12.4 Social grouping

Aggression among wild-stock is generally similar to that shown by the more aggressive laboratory strains. Isolation of males for more than a few days is likely to reduce their social tolerance considerably and unfamiliar adult males should not be housed together. Males housed together prior to puberty can live relatively peacefully, but aggression can suddenly escalate and even well-established groups must be continually monitored.

Females show a strong preference for living and nesting in small groups. Non-breeding females generally show little aggression but breeding females can be highly aggressive towards intruders of either sex. Isolated females often show persistent attempts to escape even after many months and it is recommended that non-breeding stock are only housed singly with good reason. Social disruption and poor breeding performance can all be sparked off by human disturbance or by the presence of unfamiliar personnel.

Recommendations:

- **Do not use wild mice unless it is absolutely essential.**
- **Wild mice should always be properly quarantined and treated before being housed in the same unit as laboratory strains. Avoid any possible cross contamination by staff.**
- **Animals should be maintained under reverse lighting conditions. They should be approached and handled under red light.**
- **Non-breeding stock females should be group housed unless there is a very good reason not to do so.**
- **Unfamiliar adult males should not be housed together and even well-established groups of males must be continually monitored.**
- **Avoid leaving holes for water bottle spouts in cages unplugged, and do not use mesh 10 mm or wider.**
- **Suitable shelter and nest materials such as shredded paper tissue should always be provided.**
- **Food intake should be carefully monitored over the first few days in captivity and mice gradually weaned on to laboratory diets if necessary.**
- **Whenever possible, wild mice should be handled indirectly. Nest boxes or tunnels with sliding doors or covers provide a stress-free method for catching and moving mice around.**
- **Access to areas housing wild mice should be restricted to trained staff.**

13 Towards an ideal system: research areas

Ideally, animals should have sufficient good quality structured space to enable them to display a range of different natural behaviours. This cannot be provided by existing standard unenriched laboratory cages. The information presented in this report shows that there is still a lot of research necessary to establish how current systems could be modified to satisfy the physiological and psychological needs of mice in the laboratory situation. There is also an urgent need for scientific evaluation of alternatives to current systems.

Stauffacher (1994) proposes an ethological concept for the development of laboratory animal housing which meets the animals' basic requirements and this is a concept the Working Group supports. There is *prima facie* evidence that at least 'nest box' or nesting provision is important for mice and the ease of nest-building should be incorporated into cage design. There are a number of other areas where research is essential to assist in designing a better system. These are listed below.

Recommendations for research areas:

- ***Cage size.*** **An optimum practical size for a cage, that will substantially meet the needs of mice whether singly or group-housed and with respect to sex, breed and strain, should be established.**
- ***Cage material and floors.*** **The preferences of mice for plastic or metal cages in conjunction with the different floors used needs to be examined. The type of floor also needs to be evaluated in conjunction with different bedding and nesting materials. The incidence of disease and injuries on different floors and their effect on behaviour should also be investigated.**
- ***Cage cleaning.*** **There is a need for a definitive study to determine the most appropriate clean out strategy for males, females and breeding groups. Behaviour and levels of aggression in different clean-out systems need to be examined. The use of tray liners and flushing systems, should be assessed.**
- ***Lighting regime.*** **The most appropriate lighting regime, including the use of red light, needs to be investigated. Particular attention should be paid to albino strains.**
- ***Assessing welfare.*** **Ways of assessing welfare in mice should be investigated since choice tests have limitations.**
- ***Cage inclusions.*** **The benefits of different sorts of cage inclusion for different strains of mice needs to be investigated, together with their practical application in different management systems. The possibility of developing cages with in-built baffles and barriers as an integral part of cage structure could also be studied.**
- ***Containment systems.*** **The consequences of containment systems for mouse welfare needs immediate investigation.**

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