Use of score sheets for welfare assessment of transgenic mice

M. van der Meer[1], A. Rolls[1], V. Baumans[1], B. Olivier[2,3] & L. F. M. van Zutphen[1]

[1]Department of Laboratory Animal Science, Utrecht University, PO Box 80.166, 3508 TD Utrecht, The Netherlands, [2]Department of Psychopharmacology, Utrecht University, PO Box 80.082, 3508 TB Utrecht, The Netherlands and [3]Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508, USA

Summary

The use of transgenic mice has increased dramatically in recent years and continues to increase further. However, because transgenesis may alter a balanced genotype and produce unpredictable effects, careful monitoring of health and welfare of the transgenic animal is advised. The present study assessed the feasibility of the use of score sheets for monitoring transgenic mice, as part of daily routine, in a transgenic unit. The score sheets used were based on parameters which are sensitive and easy to determine. The score sheets were used by two animal technicians and a thorough evaluation showed that the score sheets, as described in this paper, are useful for routine monitoring in a transgenic unit and may result in the early detection of animal welfare problems. However, notwithstanding the limited number of parameters included and the restricted age-span covered by the screening, the monitoring system was considered to be time consuming. Large-scale implementation of such a scoring system during the first weeks of life would increase daily care time by at least 15–20 min for an average litter of 4–6 pups. Nevertheless, the use of score sheets seems to be a prerequisite for monitoring the animal's welfare in the course of producing transgenic lines.

Keywords Score sheets; health; welfare assessment; transgenic mice

The development of transgenic and targeted mutant (knockout) technologies over the past decade, has led to a rapid increase in the number and varieties of genetically modified mice. However, the effects of genetic manipulation are still incompletely understood, and the characteristics of transgenic animals are frequently found to differ from those anticipated. This is caused by limitations in the control of the insertion of the DNA, which are inherent in some techniques (e.g. microinjection). In other situations it is attributed to the unexpected interaction of the introduced DNA with other genes. These interactions vary with the genetic background, as has frequently been observed with mice (Gordon 1997). Thus, transgenic technology can alter a balanced genotype and produce unpredictable and unexpected effects. Interfering with the genotype by inserting or removing fragments of DNA may result in a drastic alteration of the animal's normal genetic homeostasis, which can be manifested in the behaviour and well-being of the animals in unpredictable ways (Cockayne *et al*. 1994, Ting *et al*. 1994, Costa 1997). Uncontrolled expression of inserted genes may result in an increase in morbidity

Correspondence to: Miriam van der Meer
E-mail: m.vandermeer@las.vet.uu.nl

Accepted 28 June 2001

and mortality, frequently a problem encountered through using microinjection of multiple copies of a gene and through the resulting overexpression or overproduction of the gene product (Mepham *et al.* 1998). Replacement or disruption of functional genes with non-functional counterparts (targeted mutagenesis/knockouts) results in a failure to produce a functional gene product. This technique has been applied in mice to create models of human and animal pathology or disease. In situations where pathology is established, it is likely that animals suffer to some degree (Mepham *et al.* 1998).

The unpredictable and uncontrollable nature of the production techniques used for making transgenic mice indicate a need for more care for the animals after the production has been completed to ensure a reasonable quality of life. Animal care-givers are now faced with the difficult task of developing general guidelines for assess and to ensure the welfare of these animals. Despite the relatively long history of the science of genetic manipulation, there is, at the moment, no European legislation specifically concerning the production and use of transgenic animals. There are some directives which cover their use, for example, Directive 86/609/EEC regulating the use of animals in experiments and testing, and the directives on genetically modified organisms (GMOs). However, the implementation of these directives on GMOs, within national laws, is left to each individual European Union (EU) Member. This means that the extent of protection and care for transgenic animals varies among EU Members. For example, some countries may define continued breeding of an established transgenic strain as an experiment, whereas other countries may not, leaving the welfare of future generations of mice unprotected.

In The Netherlands, the generation and maintenance of a transgenic strain requires approval from the Committee on Animal Biotechnology on top of the regular approval by local Animal Care and Use Committees. Also, the Inspectorate demands that researchers keep an 'animal welfare assessment diary' for every transgenic strain they use. However, the definite content of this diary has not been specified (Code of Practice 2000). Any welfare assessment of transgenic mice should include not only identification of 'intended' adverse effects (as for animal models of disease), but also the general monitoring of parameters that may be considered to be indicators of the well-being of the animals. The unpredictable nature of non-intended effects, such as those caused by insertional mutations and genes of unknown or hypothetical function, may then also be covered. This welfare assessment scoring not only needs to be repeated for every single newly produced strain of transgenic animals, but it should also be a continuous monitoring, as not all effects of the transgene will necessarily be apparent immediately. For the welfare assessment to be useful on a routine basis it should lead to an 'as early as possible' detection of impaired well-being. When the assessment indicates that welfare is impaired, measures should be taken to alleviate the suffering; for example, feeding regimen or housing situation could be changed. In this way, the group of animals being monitored will itself directly benefit, as will future generations of transgenic mice.

Many criteria for the quality of welfare of animals have already been put forward (Morton & Griffiths 1985, Broom 1986, 1991, Barnard & Hurst 1996, Morton 1997, Rowan 1997), including physiological effects (e.g. growth, reproduction, longevity, immune suppression, corticosteroid levels, disease, injury) and behavioural responses (e.g. preferences, stereotypies, anxiety). However, it is impractical to include them all in any programme of routine monitoring because of time- and labour-related constraints. Therefore, the challenge is to select measurable biological parameters that will cover most of these criteria, while enabling the monitoring of a large number of animals. Recently, a number of methods have been explored for monitoring the health and welfare of transgenic mice using score sheets (Morton 1998, MUAWC 1999, Mertens & Rülicke 1999, 2000). In those cases the scoring has been (or will be) performed by researchers on a separate experimental basis and is not incorporated into the existing

routines of animal care, or involves a very large number of parameters.

We have developed a scoring system, containing a limited number of sensitive, easy to determine and non-invasive parameters, selected from our previous studies on implications of transgenesis for the well-being of mice (van der Meer *et al*. 1999, 2001a,b,c). This scoring system has been tested, to assess the use of the score sheets for monitoring the welfare of transgenic mice on a practical basis, as part of the animal technicians' daily routine in a transgenic unit. The score sheets include both the pre- and post-weaning period of the mice. Screening mice from birth allows any innate deficiencies to be identified and quantified at an early stage of life, rather than waiting for such effects to become manifest at a later stage (Costa 1997). Days 0 to 6, 10 and 14 were selected, based on expected developmental progress (Baumans 1999, van der Meer 1999). Up to 14 days the monitoring used mainly developmental parameters provided by a guideline with the features and behaviours which are expected in normal developing mice. The score sheets from weaning onwards included more behavioural parameters (van der Meer 2001a).

Survey design

Three score sheets were developed, two for the pre-weaning period (sheets A and B) and one for the weaning and post-weaning period (sheet C). The score sheets were based on quick and easy scoring, in consequence of which the number of tests involving individual handling procedures was kept to a minimum. The score sheets required mainly yes/no answers. No invasive techniques were included; only observations and basic handling. The monitoring lasted from birth up to 5 weeks of age. Two animal technicians of the Central Laboratory Animal Institute, Utrecht University, performed the scoring. Fifteen B6D2F$_1$/CrlBR (Charles River, Sulzfeld, Germany) foster mothers were used as recipients for the genetically manipulated egg cells. Monitoring of 64 pups from three newly produced transgenic strains occurred at the following developmental stages: days 0 through 6, day 10, day 14, at weaning (week 3), one week after weaning (week 4) and 2 weeks after weaning (week 5). For the first 6 days after birth the animals were not scored individually (to avoid possible cannibalism by the foster mother due to disturbance of the nest). The scoring was based on survival and food intake (milk spot) by indicating how many pups qualified for each category (score sheet A). It was not necessary to remove the pups from the cage during monitoring. The next stage of development was monitored on day 10 (score sheet B), at which stage the pups were individually marked and scored on survival, morphological and sensorimotor development. Scoring on day 14 was similar to that on day 10, but two extra parameters were added, namely whether the eyes and ears were open or closed. The same score sheets were used at weaning as well as at post-weaning, at 4 and 5 weeks of age. These sheets are slightly more complicated, as frequently more options are available than just yes or no. The animals were scored on survival and developmental factors, and also on behavioural characteristics and/or abnormalities and signs of ill health (score sheet C).

The objectivity of the test was assessed by scoring some of the same litters ($n = 5$) by both technicians. In addition, during data collection, the technicians provided a verbal evaluation of the score sheets and filled out a multiple choice evaluation form afterwards. This consisted of four parts containing several questions such as time (estimation of time needed, possibility to implement the procedure in the daily routine), clarity (of score sheets and observations), disturbance (of mother and pups) and general comments/criticism (opinion on the use of such scoring sheets, and whether parameters were missing).

Results and discussion

The main goal of the study was to examine the general feasibility and usefulness of the score sheets and to determine whether animal technicians were able to perform the monitoring as part of the daily routine.

Ⓐ Score sheet day 0 – day 6

DNA construct:_____.
Transgenic technique: microinjection /ES-cells
Date of birth:_____

Foster mother:_____.
Expected effects :_____.
Litter size at birth:_____.

Animal technician_____

DAY	0	1	2	3	4	5	6	Comments
Dead[1] date								
cannibalism								
Pups in nest[2]								
Pups out of nest[3]								
Milkspot[4]								
Abnormalities[5]								

[1] Number of dead pups. In the case of cannibalism place a "+" in the box marked "cannibalism".
[2] Fill in the number of pups in the nest
[3] Fill in the number of pups out of the nest
[4] Fill in the number of pups that clearly have milk in their stomachs
[5] Any distinct traits or abnormalities can be recorded here

Ⓑ Score sheet day 10/day 14

DNA construct:_____.
Transgenic technique: microinjection / ES-cells
Animal technician:_____.

Foster mother: _____
Date of birth: _____
Date: _____

INDIVIDUAL MICE	1	2	3	4	5	6	7	Comments
Identification mark[1]								
Dead[2]								
Cause of death								
In the nest[3]								
Weight (grams)								
Fur growth[4]								
Nipples[5]								
Upper incisors[6]								
Lower incisors[7]								
Walking[8]								
Righting[9]								
Ears open[10]								
Eyes open[11]								

[1] Fill in each animal's identification mark (for example: number, colour, ear clip etc)
[2] To be completed if the animal is dead, if known, fill in the cause of death.
[3] + = the animal is in the nest, - = the animal is not in the nest
[4] + = covered with soft fuzzy fur, - = little or no fur / bald patches.
[5] + = nipples are clearly visible, - = no nipples visible.
[6] + = upper incisors fully erupted, - = upper incisors not fully erupted.
[7] + = lower incisors fully erupted, - = lower incisors not fully erupted.
[8] + = mouse shows mature locomotion, stable and supported by all four limbs, - = any deviation.
[9] The animal is laid on its back. + = the animal immediately turns over onto all four limbs, - = any deviation.
[10] + = ears are open, - = ears are not yet open (only scored at day 14).
[11] + = eyes are open, - = eyes are not yet open (only scored at day 14).

Ⓒ Score sheet at weaning and after weaning

DNA construct:_____. **FM:**_____.
Transgenic technique: microinjection / ES-cells **Date of birth:** _____.
Animal technician:_____ **Date:**_____.

INDIVIDUAL MICE	1	2	3	4	5	6	7	Comments
Dead[1] cause of death								
Identification mark[2]								
Posture[3] normal								
huddled up								
other abnormal position								
Reaction to cage opening[3] active (normal)								
hyperactive								
little/no response								
Reaction to handling[3] none (normal)								
irritation/biting								
fear (faeces/urine)								

[1] To be completed if the animal is dead, if known, fill in the cause of death.
[2] Fill in each animals identification mark (for example: number, colour, ear clip).
[3] Select one of the three possibilities and mark it with a "+".

Continuation: Ⓒ Score sheet at weaning and after weaning

Date: _____.

Individual mice	1	2	3	4	5	6	7	Comments
Weight (grams)								
Gender (♂/♀)[1]								
Fur[2] normal[3]								
pilo-erection								
bald								
other abnormalities								
Whisker chewing (+/-)[4]								
Abnormalities to eyes (+/-)[5]								
Walking[6] normal								
circles								
unable to stand on 4 legs								
other abnormalities								
Aggression after cleaning (+/-)[7]								
Unexpected phenotype								

[1] Fill in ♂= male, ♀= female.
[2] Select one of the four possibilities and mark it with a "+".
[3] Fur normal = smooth and shiny.
[4] + = confirmed/present, - = absent.
[5] + =abnormal eyes for example; red, closed, excessive tear production, - = no abnormalities.
[6] Select one of the four possible answers and mark it with a "+".
[7] + = animal is aggressive towards cage mates after cleaning,. - = animal shows no aggression.

Time element

The evaluation form showed that completing the score sheets was time consuming and could not generally be incorporated into the existing work routine in the transgenic unit, as there were approximately 4000 mice present at the time. However, the animal technicians agreed that welfare monitoring of transgenic mice should be performed and that they were the persons best qualified for this job. It seems likely that the time required will be less when the scorers have become more experienced. However, monitoring should still be considered to be an additional task on top of the regular workload. As such, extra time and funds should be made available if it is to be implemented successfully.

Use of the score sheets

Throughout the monitoring procedure, the animal technicians filled in the score sheets independently and with no extra guidance. They considered the forms to be clear and concise, and all the observations and tests easy to perform. The technicians would have liked to have seen more possible answers on the score sheets than just yes (+) or no (−). Extending the range of answers with +/− for the developmental parameters of sheet B (fur; nipples; incisors) is recommended. Further differentiation, however, could increase subjectivity and is more time consuming. A high level of objectivity is important, as the aim is to create score sheets that can be filled in by a variety of scorers. For practical use as part of the daily routine, it is important that more technicians are qualified for the monitoring, especially in large laboratories with varying working schedules. It is difficult, though, to avoid personal interpretation completely, particularly on behavioural parameters. When both technicians scored the same litters independently, all parameters were scored identically, except for some differences for the scores on reaction to handling and cage opening (sheet C). Although some variation can be expected, it is our feeling that, in cases where more technicians are involved in the scoring, some instructions in advance on how to interpret a given observation may prove beneficial in reducing the individual variation among scorers. Although it can be expected that experienced technicians may recognize many of the signs depicted on the score sheets, the scoring system presented is also very useful for training newer and less experienced research staff and animal technicians, as it will help them in how to observe the animals and what to observe in them.

In order to save time, presenting the score sheets in the form of a log-book with a front cover sheet, where previous scoring days can be seen, was recommended. The time-consuming job of filling in general information could then be cut to a minimum. On the current sheets date of birth, DNA construct, name of scorer and foster mother had to be filled in repeatedly, on every score sheet.

Different score sheets were used, during both the pre- and post-weaning period. Monitoring of pups on days 0 through 6 was not very time consuming (< 5 min for one average litter of 4–6 pups) and the disturbance levels for both foster mothers and pups were considered to be acceptable. However, the strain of the (foster) mother used is important. The foster mothers monitored here ($B6D2F_1$) are rather docile compared to some of the other strains used. In more nervous strains, disturbing newly-born litters of pups could result in cannibalism or neglecting of the pups by the mother. Also when monitoring further breeding of transgenics, aspects like the strains of females and the use of primi- or multiparous females (Baumans 1999) are points for consideration. It was possible to incorporate the initial scoring into the daily schedule, as new litters had already been routinely checked for number of pups and pup viability. Days 10 and 14 scoring took some more time as the mice had to be individually marked (approximately 5–10 min for one average litter of 4–6 pups), but it could also be incorporated into the schedule.

Traditionally, weaning is a disturbing activity for young mice, especially when their tail tip has to be removed for DNA detection and an ear clip has to be performed for identification purposes. It was thought that the extra tests involved in monitoring

welfare at weaning did not increase the disturbance or discomfort level. They did, however, increase the amount of time spent on the weaning procedure, especially when large litters were involved (approximately 10–15 min for one average litter of 4–6 pups). Testing of the animals routinely after weaning involves a lot of extra work (approximately 15–20 min/litter) for the animal technicians and this cannot readily be incorporated into the existing daily schedule. Usually, the mice are only observed superficially when the cages are cleaned. Now, every animal had to be examined and weighed individually. The weighing, in particular, was time consuming. However, depressed weight gain in young animals or abnormal weight loss in adults is an extremely useful indicator of poor welfare (Broom 1993). Therefore, although it is time consuming and maybe stressful to the animal (each mouse must be individually handled), it is our feeling that this aspect cannot be abandoned. Furthermore, the handling itself has an important function as it draws the technicians' attention to any other problems that are not specifically mentioned on the score sheet and might be missed otherwise, like hypothermia or the presence of tumours. Moreover, the animal's reaction to the handling is also an important behavioural parameter. Information on the expected phenotype of transgenic mice should be made available to the animal technician, in order to be able to monitor the animals more carefully.

Parameters used

From the evaluation form it was shown that the animal technicians did not feel that any parameters were missing from the score sheets. There were some suggestions for changes concerning the make-up of the current score sheets. For example, they felt some parameters could be left out. Whisker chewing is not very common and thus could be discarded on the general form and added as a comment when it occurs. The same goes for cannibalism. It is difficult, though, to know where to draw the line. On the one hand the observations must be simple, quick and non-invasive, but on the other hand they must be effective and provide information that is relevant for animal welfare considerations. There must also be sufficient different parameters so as to make statements concerning welfare possible. A single indicator could show that welfare is poor, but absence of an effect on an indicator does not necessarily prove that welfare is good (Broom 1993). By leaving certain parameters out it is possible that these factors will be overlooked on the few occasions that they occur. The same argument holds for the possibility of having the technicians only filling in the answers that are 'unusual' or 'unexpected'. This would most likely significantly lessen the amount of time spent on monitoring. It would, however, also make it impossible to tell whether monitoring had taken place at all and will most likely lead to less precise work as the mice would not be routinely screened. Furthermore, it would rely strongly on personal observations, as the scorers would have to note that 'something is wrong'. This would defeat the purpose of routine monitoring, as the idea is that routine monitoring will effectively take over the job of identifying problems.

It is of utmost importance that the score sheets are simple, so as to minimize the time needed to complete them (see also Lloyd & Wolfensohn 1999, Lloyd *et al*. 2000). By introducing more parameters and/or more possible answers the sheets would become more complicated and the time needed to complete them would increase. Further research to determine the functionality of the score sheets should help decide whether extra measurements and observations are actually necessary.

The score of the milk spot proved to be particularly predictable as an indicator of pup survival. All pups with a visible milk spot throughout the first week survived, whereas pups not meeting this criterion died. It is important to observe whether only an individual pup is affected by lack of stomach-filling (possibly transgene-related), or an entire litter, indicating a putative maternal effect, like absence of milk or poor mothering (Mertens & Rulicke 1999, Lloyd *et al*. 2000, van der Meer *et al*. 2001b). Therefore, stomach-filling is not only useful for individual monitoring, but also gives

important clues about specific cause(s) of possible breeding problems (Mertens & Rulicke 1999).

Further monitoring

The score sheets described in this study proved to be feasible for routine monitoring in a transgenic unit, provided that the additional time needed for the observations, funds and well-instructed technicians are made available. However, there are some points that would further increase the value of the monitoring system. The present study included only the first 5 weeks of life of newly produced transgenic mice. Score sheets are also useful for monitoring the further breeding of transgenic lines. This monitoring should last from birth until the death of the animal (whether spontaneous or caused by euthanasia at the end of the experiment), for at least two generations (Broom 1997). Elements like mating behaviour, gestation, rearing of the young, milk production and the number of pups from the next generation that reach weaning age could shed a light on the reproductive success of the transgenic mother and thus on the viability of the strain.

More elements of social interaction would also be interesting to add. They may be difficult to observe, though, as the animals are likely to be disturbed by the investigator's presence or may hide in the nest. Testing more behavioural parameters by the animal technicians is rendered impractical by the time element. However, to increase the behavioural phenotyping of newly produced transgenic and mutant mice and to improve the animal welfare assessment, additional behavioural tests may be performed by the researcher. Several behavioural studies for the validity of the new mouse models have already been performed (Crawley & Paylor 1997, Crawley 1999, Rogers *et al.* 1999, Brown *et al.* 2000). In our previous studies on the implications of transgenesis for the well-being of mice, we have described some non-invasive and easy to determine behavioural tests, which were found to be discriminative for the detection of significant differences in behaviour (van der Meer *et al.* 1999, 2001b,c). These tests include, among others, pre-weaning behavioural tests, the hole board test (exploration and habituation), the cage emergence test (reaction to novel environment), the light–dark test (index of anxiety), circadian rhythm (by using the automated LABORAS device), climbing behaviour (adding a climbing object as cage enrichment) and response to handling (at and after handling in the home cage). Such a battery of tests could be used for the careful monitoring of health and welfare of transgenic offspring.

An alternative to the time-consuming weighing of the animals might be body condition scoring (BCS), which is a useful, rapid and practical tool for evaluating overall condition and health assessment of the mouse (Foltz & Ullman-Cullere 1999). It can be scored during weekly cage cleaning, when the animals have to be handled anyway. BCS is particularly helpful in cases where pregnancy, organomegaly, or tumour growth may interfere with body weight assessment. In short, the mouse is picked up by its tail and the body condition is noted by passing a finger over the back bones, while, according to some observers, just looking at the animal will also give a quick indication. The body condition can be scored on a scale of 1–5; score 5 being obese without the ability to feel the back bones at all, while with score of 2 up to 1, the mouse is becoming thin, the bones are prominent and muscle wasting is advanced. A body condition score of 2 or 1 suggests a decline in overall condition and euthanasia is recommended. Considering BCS score and weight loss may be useful. A weight loss of 10–15% within a few days or an overall weight loss of 20% are criteria for euthanasia (Foltz & Ullman-Cullere 1999).

Finally, it is important to consider the inclusion of postmortem parameters in monitoring, such as general macroscopic inspection, the weighing of several organs (e.g. heart, kidney, liver, spleen), and further microscopic examination, when necessary, at the end of the experimental period, in order to evaluate the consequences of the introduction or knockout of genes for the well-being of the animals (van der Meer *et al.* 2001c). The information gained by postmortem inspections can provide clues as to

how the *in vivo* parameters should be interpreted as indicators for animal welfare.

Dealing with welfare problems

Whenever there are obvious indicators of welfare problems detected during the routine monitoring (e.g. behavioural, clinical or morphological), the animal welfare officer or veterinarian in charge should be notified. They are the persons to decide whether euthanasia or increased monitoring of the animal(s) is required. The score sheets form the basis for taking action with respect to welfare and will be a valuable tool for all persons working with the animals. When welfare is likely to be compromised for one or more animals, this can be easily visualized for other scorers and the researcher by using differently coloured labels on the cage, with the date of scoring on the back. The necessity of increased monitoring of the animal(s), for example on a daily basis, could also be implicated by using this system (W. Kort, personal communication).

The welfare assessment should be considered to be an integral part of transgenic procedures and, by including the recording of all abnormal observations in international databases and journals, it could contribute towards refinement in transgenic technology, avoiding the use or wastage of extra animals. In this way subsequent users could be informed of adverse effects, how to recognize them, and which action to take.

Conclusion

During this study it has become clear that monitoring the welfare of transgenic mice using score sheets is both practical and useful. However, under the present circumstances in standard animal facilities, sufficient time in the animal technicians' daily schedule is the stumbling block. Therefore, the usefulness of the score sheets would not be increased by broadening the range of parameters. However, some parameters can be left out and/or exchanged by other parameters, depending on the specific characteristics of the mutant or strain. Subjective input can be reduced to a minimum by education of the scoring personnel.

The design of the score sheets needs to be flexible so they remain effective after the introduction of any changes. Animal technicians need to be instructed on how to employ the scoring systems to the animals' best advantage, and specific guidelines must be set up to indicate how the results should be interpreted. Great care must be taken in instructing scorers as to the relevance of their results. Humane endpoints must be established, i.e. the point at which animal welfare has reached such a poor level that the experiment should end and the animals should be euthanased. In this way, monitoring the welfare of transgenic animals will provide a way of minimizing animal distress. The score sheets described in this study can be used for routine monitoring and can thus, it is hoped, contribute to the design of the welfare assessment diary. They can also be very useful for training newer research staff and animal technicians in determining what to look for.

Recommendations

The following recommendations on the use of score sheets can be made based on this study:

- The practical value of the score sheets, as predictors of impending death as well as indicators of poor welfare, needs to be further confirmed in wider situations and subjected to retrospective analysis.
- Monitoring the welfare of transgenic mice using score sheets should be introduced on a routine basis.
- Animal technicians should perform the monitoring.
- Extra time should be made available in the animal technicians' timetables to include welfare monitoring.
- Screening should take place during both the pre- and post-weaning period.
- After weaning, the scoring can take place once a week during cage cleaning. Scoring parameters should be adjusted to the specific characteristics of the strain.

- Whenever obvious welfare problems are detected, the animal welfare officer or veterinarian in charge should be notified. Increased monitoring or euthanasia might be necessitated.
- The number of parameters on the score sheets should be kept to the minimum needed for the purpose of the scoring.
- For most of the parameters, the number of possible answers should be limited to a maximum of three (+; −; and +/−).

Acknowledgments The authors are grateful to animal technicians Toon Hesp and Herma Boere for careful scoring and for evaluating the scoring system. This research was financially supported by a grant from Solvay Pharmaceuticals, BV, Weesp, The Netherlands.

References

Barnard CJ, Hurst JL (1996) Welfare by design: the natural selection of welfare criteria. *Animal Welfare* 5, 405–33

Baumans V (1999) The Laboratory Mouse. In: *The UFAW Handbook on the Care and Management of Laboratory Animals, 7th edn. Volume 1: Terrestrial Vertebrates* (Poole T, ed). Oxford: Blackwell Science, pp 282–312

Broom DM (1986) Indicators of poor welfare. *British Veterinary Journal* 142, 524–5

Broom DM (1991) Animal welfare: concepts and measurement. *Journal of Animal Science* 69, 4167–75

Broom DM (1993) Assessing the welfare of modified or treated animals. *Livestock Production Science* 36, 39–54

Broom DM (1997) Assessing the welfare of transgenic animals. In: *Welfare Aspects of Transgenic Animals, Proceedings of EC-workshop, 30 October 1995, Utrecht, The Netherlands* (van Zutphen LFM, van der Meer M, eds). Berlin: Springer Verlag, pp 58–67

Brown RE, Stanford L, Schellink HM (2000) Developing standardized behavioral tests for knockout and mutant mice. *Institute for Laboratory Animal Research (ILAR) Journal* 41, 163–74

Cockayne DA, Bodine DM, Cline A, Nienhuis AW, Dunbar CE (1994) Transgenic mice expressing antisense interleukin-3 RNA develop a B-cell lymphoproliferative syndrome or neurologic dysfunction. *Blood* 84, 2699–710

Code of Practice: Welzijnsbewaking van Proefdieren (2000) Keuringsdienst van Waren, Inspectie W&V, Zutphen, The Netherlands

Costa P (1997) Production of transgenic animals: practical problems and welfare aspects. In: *Welfare Aspects of Transgenic Animals, Proceedings of EC-Workshop, 30 October 1995, Utrecht, The Netherlands* (van Zutphen LFM, van der Meer M, eds). Berlin: Springer Verlag, pp 68–77

Crawley JN (1999) Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. *Brain Research* 835,18–26

Crawley JN, Paylor R (1997) A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Hormones and Behavior* 31,197–211

Foltz CJ, Ullman-Cullere M (1999) Guidelines for assessing the health and condition of mice. *Lab Animal* 28, 28–32

Gordon JW (1997) Transgenic technology as an alternative to animal experimentation. In: *Animal Alternatives, Welfare and Ethics* (van Zutphen LFM, Balls M, eds). Amsterdam: Elsevier Science, pp 95–112

Lloyd MH, Wolfensohn SE (1999) Practical use of distress scoring systems in the application of human endpoints. In: *Humane Endpoints in Animal Experiments for Biomedical Research, Proceedings of the International Conference 22–25 November 1998, Zeist, The Netherlands* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 48–53

Lloyd M, Wolfensohn S, Thornton P (2000) Quantitative assessment of welfare in experimental animals: the development and use of scoring systems. In: *Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Science: Progress in the Reduction, Refinement and Replacement of Animal Experimentation, 29 August to 2 September 1999, Bologna, Italy* (Balls M, van Zeller A-M, Halder ME, eds). Amsterdam: Elsevier Science, pp 1107–17

Mepham TB, Combes RD, Balls M, Barbieri O, Blokhuis HJ, Costa P, Crilly RE, de Cock Buning T, Delpire VC, O'Hare MJ, Houdebine L-M, van Kreijl CF, van der Meer M, Reinhardt CA, Wolf E, van Zeller A-M (1998) Report and recommendations of ECVAM Workshop 28: The use of transgenic animals in the European Union. *Alternatives to Laboratory Animals (ATLA)* 26, 21–43

Mertens C, Rülicke T (1999) Score sheets for the monitoring of transgenic mice. *Animal Welfare* 8, 433–8

Mertens C, Rülicke T (2000) Phenotype characterization and welfare assessment of transgenic rodents (mice). *Journal of Applied Animal Welfare Science* 3, 127–39

Monash University Animal Welfare Committee (MUAWC) (1999) Draft policy for the monitoring of transgenic animals. http:/www.monash.edu.au

Morton DB, Griffiths PHM (1985) Guidelines for the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* 116, 431–6

Morton DB (1997) A scheme for the recognition and assessment of adverse effects in animals. In: *Animal Alternatives, Welfare and Ethics* (van Zutphen LFM, Balls M, eds). Amsterdam: Elsevier Science, pp 235–40

Morton DB (1998) Criteria for the estimation of pain and distress in animal experimentation using score sheets of clinical signs. In: *Versuchstierkunde: Mittler Zwischen Forschung und Tierschutz. Proceedings der 36. Wissenschaftlichen Tagung der Gesellschaft für Versuchs-tierkunde GV-SOLAS.* Hamburg-Eppendorf, Germany, pp 19–27

Rogers DC, Jones DNC, Nelson PR, Jones CM, Quilter CA, Robinson TL, Hagan JJ (1999) Use of SHIRPA and discriminant analysis to characterise marked differences in the behavioural phenotype of six inbred mouse strains. *Behavioral Brain Research* 105, 207–17

Rowan AN (1997) The concepts of animal welfare and animal suffering. In: *Animal Alternatives, Welfare and Ethics* (van Zutphen LFM, Balls M, eds). Amsterdam: Elsevier Science, pp 157–68

Ting CN, Kohrman D, Burgess DL, Boyle A, Altschuler RA, Gholizadeh G, Samuelson LC, Jang W, Meisler MH (1994) Insertional mutation on mouse chromosome 18 with vestibular and craniofacial abnormalities. *Genetics* 136, 247–54

van der Meer M, Costa P, Baumans V, Olivier B, van Zutphen LFM (1999) Welfare assessment of transgenic animals: behavioural responses and morphological development of newborn mice. *Alternatives to Laboratory Animals (ATLA)* 27, 857–68

van der Meer M, Baumans V, Olivier B, van Zutphen LFM (2001a) Impact of transgenic procedures on behavioral and physiological responses in post-weaning mice. *Physiology and Behavior* 73, 133–43

van der Meer M, Baumans V, Hofhuis F, Olivier B, van Zutphen LFM (2001b) Consequences of gene targeting procedures for behavioral responses and morphological development of newborn mice. *Transgenic Research* (in press)

van der Meer M, Baumans V, Olivier B, Kruitwagen CLJJ, Van Dijk JE, van Zutphen LFM (2001c) Behavioral and physiological effects of biotechnology procedures in gene targeting. *Physiology and Behavior* 73, 41–50