

# Genetically Modified Laboratory Animals—What Welfare Problems Do They Face?

Mia Buehr and J. Peter Hjorth

*Center for Bioethics and Risk Assessment  
Department of Molecular Biology  
University of Aarhus*

Axel Kornerup Hansen and Peter Sandøe

*Center for Bioethics and Risk Assessment  
Royal Veterinary and Agricultural University, Denmark*

In this article, we respond to public concern expressed about the welfare of genetically modified (GM) nonhuman animals. As a contribution to the debate on this subject, we attempt in this article to determine in what situations the practice of genetic modification in rodents may generate significant welfare problems. After a brief discussion of the principles of animal welfare, we focus on the problem of animal suffering and review some types of gene modifications likely to cause predictable welfare problems. In this article, we also consider suffering that may be involved in the process of generating GM animals. Finally, we discuss the role of GM animals in attempts to reduce, replace, and refine the use of animals in research.

In recent years, advances in DNA technology have raised profound public concerns about the practice of genetic manipulation of organisms. Among the many questions raised in the general debate is the concern that nonhuman animals generated by the new technology be treated ethically and with consideration for their welfare. Our aim in this article is to address possible welfare problems raised by genetically modified (GM) animals. The discussion is confined to GM rodents in the laboratory, but it should be remembered that GM animals of many different species are now being generated: cow, pig, sheep, and trout among

others. The welfare of these animals also is of concern, and many of our conclusions are applicable to them as well as to laboratory rodents.

For this article, we define the term *GM* as encompassing organisms generated by (a) pronuclear injection of a DNA construct (transgenics), (b) gene targeting techniques (knockouts, knock-ins), (c) transfection with episomal vectors, and (d) genetic modification of animals by similar laboratory procedures—as distinct from those derived by conventional breeding programs or mutagenic strategies. Occasionally, these different methods of genetic modification may raise separate welfare problems; for the most part, however, all these animals can be considered together.

The fact that an animal is genetically modified by any of these procedures does not mean that it necessarily faces a welfare problem. However, a number of factors—the reproductive technologies involved in creating GM animals, unintended effects on the animal genome brought about because of the genetic manipulation, and in some cases, the genetic modifications themselves—may increase the risk of welfare problems.

In the last three decades, the study of animal welfare has become an established scientific discipline. Methods of assessing animal welfare have emerged from studies of the way in which animals react, behave, and function in different experimental and husbandry situations. Within the field, there has been some variation in the approach to animal welfare in that some animal welfare scientists emphasized health and biological functioning (Broom, 1996), whereas others (I. J. H. Duncan, 1996) held that animal welfare is primarily a matter of the feelings of the animal (suffering, pleasure). Because suffering, health problems, and impairment of biological function often accompany each other, the two approaches, in many cases, will reach the same conclusions. However, particularly in research with animals in the laboratory, it is easy to envisage situations in which the conclusions of these approaches will differ. An animal suffering from cancer at an early stage clearly has a health problem but may not yet feel pain. It is, therefore, important to state clearly what definition of animal welfare is being used (Appleby & Sandøe, 2002).

In this article, we focus on animal suffering, that is, on more or less intense unpleasant mental or physical states felt by the animal. It should be acknowledged, however, that the occurrence of such unpleasant states does not by itself imply that there is suffering. Such states are an unavoidable part of normal animal life and often serve as signals or behavioral prompts that help the animals satisfy their biological needs. Sometimes, negative experiences are compensated for by corresponding positive experiences.

Few persons would argue that a hungry animal who finds food is suffering, even though the experience of being hungry is not pleasant. Unpleasant states, therefore, represent a welfare problem only when corresponding positive feelings do not compensate them, they persist for an extended period, or they occur frequently. Thus, a captive hungry animal who is not being fed raises a welfare

problem. So too does an animal who is strongly motivated to build a nest or explore but is kept in an environment where there is no opportunity to carry out these kinds of behaviors.

The question of whether GM animals face welfare problems essentially is about whether these animals will, as a consequence of being GM, experience prolonged or frequently occurring states of pain or other unpleasant mental states.

There is a growing literature on the subject of the welfare of GM animals (Balls, 1999; Broom, 1997; Jenkins & Combes, 1999; Mani, 1998; Masood, 1997; Mepham & Crilly, 1999; Mertens & Rulicke, 1999; Moore & Mepham, 1995; Morrell, 1999; van der Meer, Costa, Baumans, Olivier, & van Zutphen, 1999). However, very few research projects designed specifically to evaluate differences in welfare requirements between GM and non-GM animals have been reported. An analysis of the welfare problems presented by a specific transgenic strain (mice expressing a bovine prion protein) was published by Jenkins and Combes (1999), but the authors did not attempt to broaden their conclusions.

Our aim in the following discussion is to outline some possible welfare problems of GM rodents and give selected representative examples. This may serve as a basis for initiatives to anticipate, prevent, or alleviate suffering in GM animals in the laboratory.

## DIFFERENCES BETWEEN GM ANIMALS AND CONVENTIONALLY DERIVED ANIMALS IN THE LABORATORY

### Predictability of Engineered Genetic Modifications

One of the strengths of GM technology is that it can, in theory, offer the possibility of “designing” a mutation and of predicting the effects of such a mutation. This is possible because most GM animals will differ in just one genetic entity from the unmodified animal, and the nature of the modification usually is known. This may enable the predicting of the phenotypic consequences of the genetic manipulation. Furukawa, Morrow, Li, Davis, and Cepko (1999) generated mice lacking *Crx*, a homeobox gene expressed in the photoreceptor cells of the eye and also in the pineal gland. As expected, the mutants displayed abnormalities of the rod and cone cells and demonstrated differences from controls in circadian activity.

In the breeding of new lines and mutations or the derivation of genetic variations using conventional mutagenesis strategies, mutant and nonmutant (wild-type) animals may differ in many genes or large segments of the genome; the investigator may have no clear idea of what or how much genetic material is involved. As a result, the consequences to phenotype cannot be predicted with accuracy.

Of course, accurate prediction of the effect of an engineered genetic modification is not always possible. Many modifications confidently expected to have major impacts on phenotype have had no detectable effect. Also, one of the principal purposes of GM work is to discover the role of a particular molecule in the determination of phenotype. If accurate prediction were possible, it would not be necessary to do the work in the first place. However, the investigator usually can make some kind of assessment of the likely outcomes of a particular genetic modification.

### Unanticipated Changes to Genotype and Phenotype

GM animals will differ from unmodified animals by virtue of specific alterations to their DNA, the details of which usually will be known to some extent. Many different kinds of alteration are possible. Sequences of native DNA may be deleted, exogenous sequences added, or existing sequences modified.

The genetic changes induced by engineered modifications usually are known in detail once a line of GM animals is established and analyzed. However, it usually is not possible to predict before the event precisely what genomic changes will result from a particular manipulation. When a DNA construct is injected into a pronucleus of a fertilized egg, it normally will integrate at random into the host genome. The investigator will not be able to control the site of integration or the number of copies of the construct that integrate, nor will the investigator be able to prevent either construct or host genome from being altered in some unpredictable manner. Even in gene-targeting strategies in which DNA constructs of known sequences replace native genomic sequences through homologous recombination, investigators do not have absolute control over the nature of the alteration they induce: deletions, duplications, truncations, and other rearrangements of both construct and host DNA are possible. The same types of changes may happen in the cells of GM animals produced by nuclear transfer from GM somatic cells to an enucleated egg (cloning). Thus, the effects of changes made to the genome of a GM animal are not entirely predictable.

The techniques of genetic engineering usually will not lead to unanticipated genome rearrangements in the cells of modified animals, but the possibility does exist. For this reason and because modifications may be made to many different genes, GM animals as a group exhibit such widely varying phenotypes that it is impossible to define a single trait common to all. Genomic alteration may result in death or in a severe phenotype with major consequences to the health of the animal. On the other hand, a GM animal may be phenotypically indistinguishable from a wild-type animal.

## Insertional Mutagenesis As a Cause of Unanticipated Phenotypes

Because a DNA construct injected into a pronucleus normally will integrate at random into the host genome, it occasionally may become incorporated into a functional gene. According to Meisler (1992), 5% to 10% of injection-transgenic animals carry such insertional mutations, and although Meisler's figures suggest that some 75% of these die before birth, there is a significant possibility that an animal may remain viable but with an unanticipated disturbance of some physiological function.

There are many examples of such insertional mutants in the literature. Males from a line of rats expressing a renin transgene (Sharpe et al., 1995) were unexpectedly infertile, and Sharpe et al. attributed these defects to the disruption by the transgene of a gene necessary for spermatogenesis. Alagramam et al. (1999) described a mutation causing deafness and circling resulting from the insertion of a transgene into a region of Chromosome 10, and many other similar examples can be found in the literature.

Gene targeting strategies generally are more precise and give more predictable results than injection of DNA into pronuclei. Targeting constructs certainly can become integrated inappropriately, become truncated, or induce deletions or rearrangements in host DNA. However, because these anomalous recombination events take place in embryonic stem (ES) cells rather than in animals and because transfected ES cells are normally well characterized before they are used to generate mice (chimaeras), these mutagenic events rarely cause entirely unexpected problems for living animals.

However, even when a gene-targeting construct is correctly inserted, the animal carrying it sometimes will exhibit an unanticipated phenotype such as that of the nerve growth factor (NGF) receptor knockouts described following. Unexpected phenotypes are, however, not confined to GM animals: They also can arise in mutagenesis, in pharmacological tests on wild-type animals, and in the breeding of spontaneous mutations.

## Welfare Considerations Related to Methods of Generation

Laboratory mice have been bred for many decades, and hundreds of mutant and inbred lines have been established. Until now, however, these animals have been bred in the conventional way with invasive procedures such as embryo transfer used only in special cases (such as the rederivation of colonies). From the point of view of some ethicists, the generation of a GM line differs significantly from

that of these conventional lines in several ways. The production of a line of GM animals involves the sacrifice of some animals and surgical procedures (i.e., vasectomies or embryo transfers) on others (van der Meer & van Zutphen, 1997). None of these procedures is unique to the generation of GM animals, but normally they are necessary for their production.

The establishment of a line by conventional breeding usually does not require these invasive procedures. However, the numbers of animals needed to maintain mutant lines or to produce congenic or recombinant inbred strains can be very large. It has been estimated (J. Bishop, personal communication, January 2003) that a recombinant inbred line may require 1,000 to 1,400 animals to establish. The derivation of a transgenic line by pronuclear microinjection, on the other hand, may require as few as 70 animals, including embryo donors, recipients, and progeny to the F2 (second filial) generation. However, the number of transgenic lines being produced today outweighs the number of recombinant inbred lines being established.

Thus, if the reduction of the numbers of animals used in research is a priority (see Discussion section), a GM line, in some cases, may require fewer animals to establish than will some conventionally derived ones. In addition, the precision with which a problem can be addressed by a suitably designed GM animal ultimately may allow fewer animals to be used to answer a specific question. Such considerations must be weighed against welfare issues raised by the invasive procedures necessary to establish a GM line.

Another aspect of genetic technology that may have implications for animal welfare is the need to take tissue samples from GM animals to determine their genotype. Normally, these samples consist of tail biopsies (tail tips taken from very young mice) or the tissue fragments generated when mice are ear punched for identification purposes. However, methods have been devised to minimize the stress and pain suffered by animals undergoing these procedures. Broome et al. (1999) used the polymerase chain reaction (PCR) to detect the product of human keratin genes in the stools of GM mice, and Schmitteckert, Prokop, and Hedrich (1999) identified transgene products in hair follicles with PCR. Such relatively noninvasive procedures conceivably could replace the more conventional reliance on tail and ear tissue. However, some investigators find that the PCR procedure needed to analyze these very small DNA samples can give unreliable results; therefore, refinements in PCR methods may be necessary before these techniques are widely used. Furthermore, studies elucidating the impact of the sampling and marking procedures are needed to find the least harmful ways to perform them.

### Phenotypic Consequences of In Vitro Methods

For some time it has been known that sheep and cattle embryos subjected to in vitro culture may develop “large fetus syndrome” in which the neonate may

weigh as much as two to five times normal weight, and this phenotype may occur even in the absence of embryo-invasive procedures (Young, Sinclair, & Wilmut, 1998). This syndrome as such has not yet been reported in rodents. Evidence presented by van der Meer, Baumans, Olivier, and van Zutphen (2001) suggests that *in vitro* procedures may result in small increases in the neonatal weight of mice, but on the other hand, humans derived from *in vitro* fertilization procedures are more likely to suffer from lower than normal birth weights (Schieve et al., 2002). The specific role of *in vitro* procedures in determining embryo size and other characteristics is, therefore, yet to be determined but may involve such factors as changes in DNA imprinting as a result of altered environmental influences (Rideout, Eggan, & Jaenisch, 2001).

Similar phenotypic alterations can result from the technique of cloning in which nuclei from somatic cells are transferred into enucleated eggs (Wakayama, Perry, Zuccotti, Johnson, & Yanagimachi, 1998). Recently, this technique was adapted for the production of GM animals by the use of donor nuclei from GM cell lines (Lai et al., 2002).

It is clear that many cloned animals have significant abnormalities. Cloned mice frequently have abnormal placentas (Tanaka et al., 2001), and only a small proportion usually survive to birth. Even survivors may exhibit abnormalities such as respiratory problems and obesity and may die prematurely (Eggan et al., 2001; Ogonuki et al., 2002; Tamashiro et al., 2002).

Generally, it is believed that these problems stem from faulty epigenetic reprogramming of the donor nucleus (Rideout et al., 2001) and thus are a specific result of the cloning procedure. Animals produced by more conventional GM techniques (i.e., injection transgenesis and gene targeting), which develop from a normally fertilized egg, would not be expected to share these problems. However, as new methods of genetic manipulation are devised and the boundaries between categories begin to blur, it is well to remember that such problems exist. The phenotype of a GM animal generated by the cloning of GM fibroblasts may prove to be very different from that of one produced by pronuclear injection.

In summary, then, GM animals differ from conventionally bred animals in several ways. Invasive procedures are necessary to establish them, and unanticipated alterations to genotype—and possibly to phenotype—cannot be ruled out.

## WELFARE ISSUES RELATED TO THE GM PHENOTYPE

### Effects of Engineered Genetic Modification on Behavior

A few attempts have been made to define some general effect of GM technology on animal behavior, but no consistent difference between GM animals and controls seems to have been reported. Of course, many lines of mice have been en-

gineered to express transgenes that are expected to modify some aspect of behavior (Cho, Friedman, & Silva, 1999; Heinrichs et al., 1997; Inui et al., 1998; Karolyi et al., 1999; Mayeux-Portas, File, Stewart, & Morris, 2000; Nelson & Chiavegatto, 2000; Rochford, Beaulieu, Rouse, Glowa, & Barden, 1997; RondiReig et al., 1997; Strohle, Poettig, Barden, Holsboer, & Montkowski, 1998; Tronche et al., 1999; Voigt, Rex, Bader, & Fink, 2000; Weiss, Lightowler, Stanhope, Kennett, & Dourish, 2000). No general differences, however, have been reported between wild-type animals and animals expressing transgenes not expected to affect behavior.

Hughes, Hughes, Waddington, and Appleby (1996) found no significant behavioral differences between non-GM sheep and sheep carrying a human gene for alpha-1 antitrypsin. Van der Meer et al. (1999; van der Meer, Baumans, et al., 2001) compared some behavioral and growth parameters of mice expressing a corticotrophin releasing factor construct and three suitable control groups. No significant differences between the groups were noted.

### Assessing the Phenotype of a GM Animal

Experience has shown that many GM animals do not present a special welfare because (a) the phenotype is unaltered in any way that affects the animal's health or equilibrium and (b) the genetic alteration prevents development to a stage where welfare is a relevant issue.

It must be remembered that GM animals and wild-type animals often react differently to experimental treatments; therefore, welfare requirements may depend on the way in which an animal is used. There are many examples of this in the literature. In a study of immune responses to *Pneumocystis carinii*, Paine et al. (2000) used GM mice lacking the cytokine granulocyte-macrophage colony-stimulating factor. When depleted of CD4 cells and inoculated with *P. carinii*, the GM animals showed a significant increase in pulmonary infection and inflammation as compared with non-GM control animals. GM mice overexpressing a subunit of the N-methyl D-aspartate receptor complex in the forebrain showed a heightened response to inflammatory pain (Wei et al., 2001).

In these examples, GM animals could be said to have suffered more ill effects than non-GM animals and therefore to be of more welfare concern than were the controls. However, GM animals also can prove less susceptible to the consequences of some experimental treatment than controls. Mice overexpressing two forms of human glutathione peroxidase were better able to survive endotoxaemia after treatment with lipopolysaccharides (Mirochnitchenko et al., 2000). In this example, as well as in many others, welfare concerns probably are most appropriately directed at the non-GM control animals.

Another concern is that a GM animal may exhibit a deleterious phenotype unrelated to the primary purpose for which the animal was generated, raising the possibility that some aspects of the phenotype may go unnoticed or that facilities may not be in place for the alleviation of possible welfare problems associated with it. Smeyne et al. (1994) produced mice lacking a functional *Trk* gene, which codes for a receptor for NGF. As might be expected, the homozygous mutants have many neurological defects, and almost all die within a month of birth. However, the mice also develop scabbing of the skin and ulcerated paws, probably due to self-mutilation; other organ systems also are affected. These aspects of the mutant phenotype might not be anticipated but could require special treatment and assessment for welfare requirements.

Finally, even if a transgene or targeted mutation is innocuous in itself, the possibility exists that it might render the animal more susceptible to conditions that would be perfectly acceptable to a nonmutant. Animals with mutations causing increased susceptibility to stress (Mirochnitchenko et al., 2000; Smeyne et al. 1994; Wei et al., 2001) or increased aggressiveness (Nelson & Chiavegatto, 2000) may require special housing and handling.

Concern about such unexpected consequences of genetic modification has led to a demand from several authors for the establishment of screens for deviations from normal phenotype, and several approaches have been suggested (Costa, 1997; Crawley, 1999, 2000; Crawley & Paylor, 1997; Foltz & Ullman-Cullere, 1999; Mertens & Rulicke, 1999; van der Meer, Rolls, Baumans, Olivier, & van Zutphen, 2001). However, there appears to be no consensus on a system suitable for monitoring deviations from normality of GM animals.

Although many GM animals are indistinguishable from wild-type animals or have an apparently innocuous phenotype, many others have been generated whose phenotypes do appear to affect health and normal equilibrium. Clearly, there is a concern about the welfare of an animal who has been engineered or bred to express a harmful mutation. A *harmful mutation* may be defined as one that damages the health of the animal after midgestation, and this definition encompasses a wide spectrum of phenotypes ranging from minor abnormalities to death. Harmful mutations raise concerns about the welfare of the animal carrying them, but there appears to be no distinction possible between a harmful mutation generated by genetic engineering techniques and one generated by mutagenesis or by breeding naturally occurring mutations.

### Assessing the Welfare Status of Animals With Harmful Mutations

In the past, lists of potential welfare problems have been compiled (Broom, 1997; Broom & Johnson, 1993) that may apply to laboratory and other animals. These in-

clude states such as (a) pain and discomfort; (b) curtailed life expectancy; (c) inability to feed; (d) sensory deprivation, such as deafness and blindness; (e) inability to mate; or (f) restrictions in reproductive or other behavior. The general conclusions of such papers are that, in the interests of welfare, animals carrying harmful mutations that might cause these states—whatever the origin of the mutation—should be assessed with regard to these potential problems.

How should animals be assessed? A commonsense approach suggests that in the case of some physical states, it may be appropriate to extrapolate from human to animal experience and assess an animal's reaction according to the reaction of a human in the same condition. Almost all people would consider it obvious that states painful to humans—prolonged hunger, thirst, or painful stimuli—will cause distress to an animal and that these states represent genuine welfare problems.

However, when it comes to less well-defined psychological, and even some physical, states, human experience may not necessarily be a suitable guide. Even conditions that in humans can cause considerable distress (i.e., deafness) may have little or no effect on the general health or psychological equilibrium of a mouse raised and maintained in laboratory conditions. It also can be argued that a genetic lesion causing a premature but painless and stress-free death need not constitute a major welfare problem. For these reasons, a condition fitting the definition of a harmful mutation does not imply necessarily a welfare problem.

Many lines of GM animals, however, do present welfare problems. Mice overexpressing protein kinase C epsilon under the control of a keratin promoter (Reddig et al., 2000) develop inflammation and ulceration of the skin at 4 to 5 months of age. Such animals require careful assessment and the identification of ways in which their welfare status can be improved, such as by terminating experiments before the age at which the harmful phenotype develops. For general welfare assessment, van der Meer, Rolls, et al. (2001) recommended a scoring system for the performance in the first 2 weeks after birth.

### Do Disease Models Require Special Assessment?

One of the greatest benefits of GM research has been the ability to generate animal models of disease, that is, animals expressing genes that cause or predispose to human disease. Such models allow the analysis of the development and effects of a disease and by providing subjects on which potential treatments can be tested, can be effective tools in clinical research. However, the welfare of animals who are “designed to be ill” and possibly suffer as a result must be considered.

It is true that in some cases, an accurate animal model of a disease known to be painful in humans or non-GM animals probably would be painful to the animal models, such as GM animals who develop arthritic conditions (Horai et al., 2000). These mice, who lack the interleukin-1 (IL-1) receptor antagonist, exhibit

joint-specific inflammation similar to that seen in some types of human arthritis; there seems to be no reason to believe that the condition is less painful in mice than in humans. Many other GM animals have been generated for the study of arthritic conditions (Lee, Khare, Griffiths, Luthra, & David, 2000; Taugog & Hammer, 1996); and a large proportion of these animals may be subject to pain. In some cases, the degree of discomfort or distress exhibited by an animal is used as a standard assay of the phenotype (Cheunsuk, Gerken, Osman, Hood, & Ladiges, 1999). Other accurate models of painful human disease, such as mice lacking IL-7 who develop ulcerative colitis—can be expected to cause discomfort to the animal (Watanabe, Ueno, Yamazaki, & Hibi, 1999).

In some cases, however, a disease that is distressing in humans appears to have a milder effect on its animal model—or even no effect at all. A well-documented example is that of cystic fibrosis GM mice. In the early 1990s, three research groups generated mice carrying mutations similar to those that cause cystic fibrosis in humans. Homozygous mutant mice from two of the lines (Colledge, Ratcliff, Foster, Williamson, & Evans, 1992; Snouwaert et al., 1992) usually died as young animals from gut perforation and consequent peritonitis. However, neither strain demonstrated the pulmonary symptoms that in humans are the major cause of morbidity and early death. Mice from the third strain (Dorin et al., 1992) had no marked symptoms of cystic fibrosis and in most ways appeared normal. Several reasons have been suggested for this discrepancy in phenotype (Smithies, 1993), but the example illustrates how GM animals carrying genetic anomalies that in humans can cause disease may not always reproduce the human phenotype. Even though animal models may not mimic exactly the symptoms of a particular human condition, they may, nonetheless, prove useful in elucidating some features of the human disease. Zahm et al. (1997) made use of the mice generated by Dorin et al. (who showed very few disease symptoms) to study the effect of a mutated cystic fibrosis gene on the clearance of mucous from the airway.

Finally, many GM disease models are designed to investigate complex multifactorial conditions such as hypertension. A particular animal may be modified in such a way (e.g., by changing a single gene affecting a condition known to be polygenic in nature) that the acute phase of a disease never develops (Kantachuvesiri et al., 1999; Mullins, Morley, & Mullins, 1996). GM animals can be of great use in analyzing these complex conditions. The ability to dissect the etiology of a human disease, to identify important contributing genes, and to distinguish genetic from environmental factors are among the main strengths of GM disease models.

Thus, severe human diseases do not necessarily require animal models with equally severe phenotypes. On the same principle, an animal model possibly might suffer greater consequences to health than would humans with the same disease. For these reasons, it is not possible to make a priori judgments of the potential welfare problems of a disease model solely on the severity of the human condition;

each model must be assessed individually. Although knowledge of the human condition may allow the investigator to anticipate some possible aspects of the model's phenotype, an accurate assessment can be done only after the animal is generated and the phenotype analyzed.

### GM-Specific Syndromes?

Some disease syndromes appear to be common in GM animals of various kinds. Several different lines of GM mice and rats exhibit a combination of gastric ulcers—colitis, proctitis, and rectal prolapse—that resemble in some ways the human condition of inflammatory bowel disease (Bhan, Mizoguchi, Smith, & Mizoguchi, 1999; Podolsky, 1997). Cataracts also have been described in several lines of GM animals.

In many cases, these phenotypes were expected. For instance, the inactivation of the growth factor transforming growth factor- $\beta$  (TGF- $\beta$ ) in the intestine of GM mice results in a chronic bowel inflammation (Hahm et al., 2001). As TGF- $\beta$  is known to have effects on both extracellular matrix and immune responses, this phenotype is not surprising. Similarly, it would be expected that the expression of some transgenes in the lens or other part of the eye might cause cataracts or other eye abnormalities (M. K. Duncan, Kozmik, Cveklova, Piatigorsky, & Cvekl, 2000; Steele et al., 2000; Tumminia et al., 2001). However, in other cases such conditions appear to be unexpected or a consequence of insertional mutagenesis. The relative frequency of these and other “syndromes” occasionally has led to speculation that, irrespective of the nature of the genetic change induced, the process of generating GM animals may cause such phenotypes.

There is no evidence, however, that techniques of genetic manipulation can in themselves lead to such effects. Complex organs such as the gut and the eye are known to require the coordinated function of a great many genes operating in a variety of systems; it is not surprising that the disruption of one or more of those genes, either intentionally or accidentally, will have consequences for gut or eye phenotype. Because complex signaling pathways and other associations of gene products exist in these and other systems, several different genetic lesions may yield the same phenotype—sometimes, perhaps, giving the impression that a GM-specific syndrome may exist.

However, the vast majority of GM lines described in the literature suffer neither from cataracts nor gut disease, which suggests that neither of these “syndromes” can be attributable to the method with which these animals were generated.

It also must be recognized that some inbred strains are prone to specific phenotypic abnormalities. If these strains are involved in the generation of GM animals, the defects may reappear in the lines derived from them. The C57BL/6 strain, commonly used in the generation of gene-targeted mice, is prone to certain

eye defects (Smith, Roderick, & Sundberg, 1994), which may account for some of the descriptions of eye abnormalities in targeted lines.

## DISCUSSION

### Specific Welfare Problems of GM Animals?

Accepting that animal welfare is a key concern when animals are used in research, we attempted to determine in what situations the practice of genetic modification in rodents may generate significant welfare problems. Ours is not the first attempt to address these questions, but few authors have succeeded in defining welfare problems specific to GM animals. Van der Meer et al. (1999; van der Meer, Baumans, et al., 2001) analyzed four groups of GM and non-GM mice in an attempt to identify specific problems associated with the GM animals but did not report significant or consistent differences in the groups studied. Van der Meer and van Zutphen (1997) pointed out that invasive procedures such as superovulation and vasectomy are necessary to establish GM lines but are not required in conventional breeding strategies. However, these procedures, not confined to the production of GM animals, may be used to many other ends.

We considered the differences between GM and non-GM animals and discussed some examples of GM animals that may raise welfare concerns. We concur with most authors in this field that no specific difference can be defined between the welfare needs of GM animals and of other experimental animals. However, it still is important to present an overview of welfare problems found in GM rodents and to focus on those types of gene modifications likely to cause predictable welfare problems.

One of the few studies to consider the welfare problems presented by a specific GM mouse line is that of Jenkins and Combes (1999), who applied the ethical score system devised by Porter (1992) to GM mice expressing the bovine prion protein. Because these mice are susceptible to prion disease, they have been used as bioassays to test for the presence of infectious bovine prion particles in foods and other substances. By analyzing every stage of the bioassays in which these mice are used and applying the criteria of the Porter system to them, Jenkins and Combes identified several areas in which refinement of procedures and improvement of welfare practices were possible. Although useful as a model, Jenkins and Combes conclusions are limited to one specific GM line and are not necessarily generally applicable. Also, it is important to recognize that because the Porter system allows some painful or uncomfortable procedures to be carried out as long as other aspects of animal care are of a high quality, it is not accepted universally among those working in the welfare field.

## Comparative Welfare Status of GM and Non-GM Animals

As we mentioned earlier, the assessment of the welfare status of an animal who carries what is by definition a harmful mutation is not always a straightforward matter. However, this problem occurs with all animals in a research environment whether they are derived from conventional breeding programs or from a GM line. A catalog of the hundreds of spontaneous mutations that occur in the mouse (Lyon & Searle, 1989) provides ample evidence that harmful mutations are not confined to mice generated by GM techniques. Indeed, often a natural mutation is phenotypically indistinguishable from that of a GM line. The spontaneously occurring mutant severe combined immunodeficiency (Bosma, Custer, & Bosma, 1983) and the engineered knockout mutants Rag-1 (Mombaerts et al., 1992) and Rag-2 (Shinkai et al., 1992) all lack functional T and B lymphocytes and, as a result, are severely immunocompromised (Muller, Kuhn, & Ranewsky, 1991). A spontaneous mutation of the mouse ank gene (progressive ankylosis; Ho, Johnson, & Kingsley, 2000) causes a type of arthritis similar to the arthropathies that develop in some GM animals (see references in the “Do Disease Models Require Special Assessment” section previously). Because in these cases, the phenotype of mutant and GM animals are almost identical—the same welfare concerns are raised—whether the phenotypes arise from spontaneous mutations or from engineered lines. One advantage in dealing with GM animals is the opportunity based on knowledge of the gene and the modification involved to anticipate possible welfare problems before the animal is produced. This cannot be done with conventionally bred or mutagenized strains.

## Redesigning Experiments With GM Mice in the Interest of Alleviating Welfare Problems

Wolf and Wanke (1997) reviewed several lines of transgenic mice expressing human growth hormone that demonstrate several different phenotypes according to the promoter used. Although some of these phenotypes are unquestionably harmful, the mice provide very useful models of such conditions as glomerulosclerosis, which are unavailable in the mouse as natural mutants. The transgenic lines provide an opportunity to test treatments for such conditions as chronic renal insufficiency at a stage before the animal suffers any degenerative changes and without the need for surgery or other invasive treatments. In this case, therefore, as long as the animal is used before symptoms develop, the welfare status of the animal is likely to be superior to that of a nontransgenic mouse subjected to surgery or chemical treatment.

## Can GM Animals Provide Reduction, Replacement, or Refinement in Animal Experimentation?

Three factors are often cited as goals—in the general interests of animal welfare—of the animal experimenters. Summarized, the goals include reduction, replacement, and refinement. The number of animals used should be reduced; where possible, animals should be replaced by other systems; and the experiments should be refined to improve the focus of the experiment. Does the use of GM animals in research contribute to these welfare goals?

Often, the question to be addressed in research with GM animals involves the effect of a specific gene alteration on the entire organism; for this reason, the goal of replacing the animal with another system may not be possible to achieve. However, the ability to harvest a tissue that expresses a particular gene product may enable an experiment to be performed largely *in vitro*. Maas-Szabowski et al. (2001) made use of an *in vitro* culture system in which fibroblasts derived from GM mice play an important part in a study of the developing epidermis. Also, Tabuchi et al. (2000) developed a colonic epithelial cell line from mice carrying a temperature-sensitive large T-antigen gene, which should be a useful tool in some studies of colonic epithelium.

Even when *in vitro* studies are not appropriate, a well-designed GM animal can add considerably to the refinement of a study; such GM animals may mean that a given scientific question can be answered with fewer animals.

However, the increasing information that is available on the genome sequence of humans (and soon of mice) will probably lead to an increase in the number of scientific questions that can be addressed only by the use of GM animals. It is important to appreciate that reduction, in this case, may not mean necessarily a fall in the absolute number of animals used in research but rather a fall relative to the number of questions asked. If increased use of GM animals can answer more important questions, this need not be viewed as a problem (Hansen, Sandøe, Svendsen, Forsmann, & Thomsen, 1999). On the other hand, it is important to keep in mind that widespread use of a certain transgenic animal who is suffering from a welfare impact will result in a large number of animals suffering from this impact. Again, this is no different from the situation within other types of genetically induced animal models such as inbred animals and mutants.

## CONCLUSIONS

The use of GM animals for research has given rise to public concern. One key concern is for the welfare of the modified animals, not least when animals are

modified with the aim of creating models of human disease. To earn public support for their work, scientists must attend to the welfare of the GM animals they use, and they should try to anticipate any possible effects of their work on the welfare of research animals. However, sometimes effects on animal welfare can be difficult to foresee; therefore, it is an important task to develop and implement tools that allow the scientists and others involved to monitor signs of suffering in a systematic way. If the animals are likely to experience intense, prolonged, or frequently occurring states of pain or other forms of suffering, ways should be found to alleviate suffering. Also, there may be a need to define endpoints where experiments will be stopped and the animals euthanized before the occurrence of any substantial and severe welfare problems. This article may be of use in focusing the attention of scientists on possible welfare problems in transgenic research.

## ACKNOWLEDGMENTS

This work was funded by the Danish Research Agency.

## REFERENCES

- Alagramam, K. N., Kwon, H. Y., Cacheiro, N. L. A., Stubbs, L., Wright, C. G., Erway, L. C., et al. (1999). A new mouse insertional mutation that causes sensorineural deafness and vestibular defects. *Genetics*, *152*, 1691–1699.
- Appleby, M. C., & Sandøe, P. (2002). Philosophical debate on the nature of well-being: Implications for animal welfare. *Animal Welfare*, *11*, 283–294.
- Balls, M. (1999). Does the use of transgenic animals raise particular welfare and ethical concerns? *ATLA-Alternatives to Laboratory Animals*, *27*(Suppl. 1), 811–813.
- Bhan, A. K., Mizoguchi, E., Smith, R. N., & Mizoguchi, A. (1999). Colitis in transgenic and knockout animals as models of human inflammatory bowel disease. *Immunological Review*, *169*, 195–207.
- Bosma, C. G., Custer, R. P., & Bosma, M. J. (1983). A severe combined immunodeficiency mutation in the mouse. *Nature*, *301*, 527–530.
- Broom, D. M. (1996). Animal welfare defined in terms of attempts to cope with the environment. *Acta Agricultura Scandinavica. Section A, Animal Science*, (Suppl. 27), 22–28.
- Broom, D. M. (1997). Assessing the welfare of transgenic animals. In L. F. M. van Zutphen & M. van der Meer (Eds.), *Welfare aspects of transgenic animals* (pp. 58–67). Berlin: Springer.
- Broom, D. M., & Johnson, K. G. (1993). *Stress and animal welfare*. London: Chapman & Hall.
- Broome, R. L., Feng, L., Zhou, Q., Smith, A., Hahn, N., Matsui, S. M., et al. (1999). Non-invasive transgenic mouse genotyping using stool analysis. *FEBS LETTERS*, *462*, 159–160.
- Cheunsuk, S., Gerken, E., Osman, G., Hood, L., & Ladiges, W. (1999). Predictive parameters of joint disease in DBA/1 transgenic mice. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *54*, B271–B275.
- Cho, Y. H., Friedman, E., & Silva, A. J. (1999). Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. *Behavioural Brain Research*, *98*, 77–87.

- Colledge, W. H., Ratcliff, R., Foster, D., Williamson, R., & Evans, M. J. (1992). Cystic fibrosis mouse with intestinal obstruction. *Lancet*, *340*, 680.
- Costa, P. (1997). Production of transgenic animals: Practical problems and welfare aspects. In L. F. M. van Zutphen & M. van der Meer (Eds.), *Welfare aspects of transgenic animals* (pp. 68–77). Berlin: Springer.
- Crawley, J. N. (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, J. N. (2000). *What's wrong with my mouse? Behavioural phenotyping of transgenic and knockout mice*. New York: Wiley-Liss.
- Crawley, J. N., & 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 Behaviour*, *31*, 197–211.
- Dorin, J. R., Dickinson, P., Alton, E., Smith, S. N., Geddes, D. M., Stevenson, B. J., et al. (1992). Cystic fibrosis in the mouse by targeted insertional mutagenesis. *Nature*, *359*, 211–215.
- Duncan, I. J. H. (1996). Animal welfare defined in terms of feelings. *Acta Agricultura Scandinavica. Section A, Animal Science*, (Suppl. 27), 29–35.
- Duncan, M. K., Kozmik, Z., Cveklova, K., Piatigorsky, J., & Cvekl, A. (2000). Overexpression of PAX6(5a) in lens fiber cells results in cataract and upregulation of alpha 5 beta 1 integrin expression. *Journal of Cell Science*, *11*, 3173–3185.
- Eggan, K., Akutsu, H., Loring, J., Jackson-Grusby, L., Klemm, M., Rideout, W. M., et al. (2001). Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 6209–6214.
- Foltz, C. J., & Ullman-Cullere, M. (1999). Guidelines for assessing the health and condition of mice. *Laboratory Animals*, *28*, 28–32.
- Furukawa, T., Morrow, E. M., Li, T. S., Davis, F. C., & Cepko, C. L. (1999). Retinopathy and attenuated circadian entrainment in Crx-deficient mice. *Nature Genetics*, *23*, 466–470.
- Hahm, K. B., Im, Y. H., Parks, T. W., Park, S. H., Markowitz, S., Jung, H. Y., et al. (2001). Loss of transforming growth factor beta signaling in the intestine contributes to tissue injury in inflammatory bowel disease. *Gut*, *49*, 190–198.
- Hansen, A. K., Sandøe, P., Svendsen, O., Forsmann, B., & Thomsen, P. (1999). The need to refine the notion of reduction. In C. F. M. Hendriksen & D. B. Morton (Eds.), *Humane endpoints in animal experiments for biomedical research* (pp. 139–144). London: RSM.
- Heinrichs, S. C., Min, H., Tamraz, S., Carmouche, M., Boehme, S. A., & Vale, W. W. (1997). Anti-sexual and anxiogenic behavioral consequences of corticotropin-releasing factor overexpression are centrally mediated. *Psychoneuroendocrinology*, *22*, 215–224.
- Ho, A. M., Johnson, M. D., & Kingsley, D. M. (2000). Role of the mouse ank gene in control of tissue calcification and arthritis. *Science*, *289*, 265–270.
- Horai, R., Saijo, S., Tanioka, M., Nakae, S., Sudo, K., Okahara, A., et al. (2000). Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *Journal of Experimental Medicine*, *191*, 313–320.
- Hughes, B. O., Hughes, G. S., Waddington, D., & Appleby, M. C. (1996). Behavioural comparison of transgenic and control sheep: Movement order, behaviour on pasture and in covered pens. *Animal Science*, *6*, 91–101.
- Inui, A., Okita, M., Nakajima, M., Momose, K., Ueno, N., Teranishi, A., et al. (1998). Anxiety-like behavior in transgenic mice with brain expression of neuropeptide Y. *Proceedings of the Association of American Physicians*, *110*, 171–182.
- Jenkins, E. S., & Combes, R. D. (1999). The welfare problems associated with using transgenic mice to bioassay for bovine spongiform encephalopathy. *Animal Welfare*, *8*, 421–431.

- Kantachuvesiri, S., Haley, C. S., Fleming, S., Kurian, K., Whitworth, C. E., Wenham, P., et al. (1999). Genetic mapping of modifier loci affecting malignant hypertension in TGRmRen2 rats. *Kidney International*, *56*, 414–420.
- Karolyi, I. J., Burrows, H. L., Ramesh, T. M., Nakajima, M., Lesh, J. S., & Seong, E. (1999). Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 11595–11600.
- Lai, L. X., Kolber-Simonds, D., Park, K. W., Cheong, H. T., Greenstein, J. L., Samuel, G. S., et al. (2002). Production of alpha-1, 3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science*, *295*, 1089–1092.
- Lee, S., Khare, S. D., Griffiths, M. M., Luthra, H. S., & David, C. S. (2000). HLA-B27 transgenic mice are susceptible to collagen-induced arthritis: Type II collagen as a potential target in human disease. *Journal of Human Immunology*, *61*, 140–147.
- Lyon, M. F., & Searle, A. G. (1989). *Genetic variants and strains of the laboratory mouse*. New York: Oxford University Press.
- Maas-Szabowski, N., Szabowski, A., Stark, H. J., Andrecht, S., Schorpp-Kistner, M., Angel, P., et al. (2001). Organotypic cocultures with genetically modified mouse fibroblasts as a tool to dissect molecular mechanisms regulating keratinocyte growth and differentiation. *Journal of Investigative Dermatology*, *116*, 816–820.
- Mani, P. (1998). Animal welfare problems concerning the use of transgenic animals. *ALTEX-Alternativen zu Tierexperimenten*, *15*, 32–33.
- Masood, E. (1997). Pressure grows for inquiry into welfare of transgenic animals. *Nature*, *388*, 311–312.
- Mayeux-Portas, V., File, S. E., Stewart, C. L., & Morris, R. J. (2000). Mice lacking the cell adhesion molecule Thy-1 fail to use socially transmitted cues to direct their choice of food. *Current Biology*, *2000*, 68–75.
- Meisler, M. (1992). Insertional mutations of “classical” and novel genes in transgenic mice. *Trends in Genetics*, *8*, 341–344.
- Mephram, T., & Crilly, R. E. (1999). Bioethical issues in the generation and use of transgenic farm animals. *ATLA-Alternatives to Laboratory Animals*, *27*(Suppl. 1), 847–855.
- Mertens, C., & Rulicke, T. (1999). Score sheets for the monitoring of transgenic mice. *Animal Welfare*, *8*, 433–438.
- Mirochnitchenko, O., Prokopenko, O., Palnitkar, U., Kister, I., Powell, W. S., & Inouye, M. (2000). Endotoxemia in transgenic mice overexpressing human glutathione peroxidases. *Circulation Research*, *87*, 289–295.
- Mombaerts, P., Iacomini, J., Johnson, R. S., Herrup, K., Tonegawa, S., & Papaioannou, V. E. (1992). RAG-1 deficient mice have no mature B and T lymphocytes. *Cell*, *68*, 869–877.
- Moore, C. J., & Mephram, T. B. (1995). Transgenesis and animal welfare. *ATLA*, *23*, 380–397.
- Morrell, J. M. (1999). Techniques of embryo transfer and facility decontamination used to improve the health and welfare of transgenic mice. *Laboratory Animals*, *33*, 201–206.
- Muller, W., Kuhn, R., & Rajewsky, K. (1991). Major histocompatibility complex class II hyperexpression on B cells in interleukin 4 transgenic mice does not lead to B cell proliferation and hypergammaglobulinemia. *European Journal of Immunology*, *21*, 921–925.
- Mullins, L. J., Morley, S. D., & Mullins, J. J. (1996). Transgenics and essential hypertension. *Journal of Human Hypertension*, *10*, 627–631.
- Nelson, R. J., & Chiavegatto, S. (2000). Aggression in knockout mice. *ILAR Journal*, *41*, 153–162.
- Ogonuki, N., Inoue, K., Yamamoto, Y., Noguchi, Y., Tanemura, K., Suzuki, O., et al. (2002). Early death of mice cloned from somatic cells. *Nature Genetics*, *30*, 253–254.
- Paine, R., Preston, A. M., Wilcoxon, S., Jin, H., Siu, B. B., Morris, S. B., et al. (2000). Granulocyte-macrophage colony-stimulating factor in the innate immune response to *Pneumocystis carinii* pneumonia in mice. *Journal of Immunology*, *164*, 2602–2609.

- Podolsky, D. K. (1997). Lessons from genetic models of inflammatory bowel disease: Acta gastro-enterol. *Belgica*, 60, 163–165.
- Porter, D. G. (1992). Ethical scores for animal experiments. *Nature*, 356, 101–102.
- Reddig, P. J., Dreckschmidt, N. E., Zou, J., Bourguignon, S. E., Oberley, T. D., & Verma, A. K. (2000). Transgenic mice overexpressing protein kinase C epsilon in their epidermis exhibit reduced papilloma burden but enhanced carcinoma formation after tumor promotion. *Cancer Research*, 60, 595–602.
- Rideout, W. M., Eggan, K., & Jaenisch, R. (2001). Nuclear cloning and epigenetic reprogramming of the genome. *Science*, 293, 1093–1098.
- Rochford, J., Beaulieu, S., Rousse, I., Glowa, J. R., & Barden, N. (1997). Behavioral reactivity to aversive stimuli in a transgenic mouse model of impaired glucocorticoid (Type II) receptor function: Effects of diazepam and FG-7142. *Psychopharmacology*, 132, 145–152.
- RondiReig, L., Dubreuil, Y. L., Martinou, J. C., Delhaye-Bouchaud, N., Caston, J., & Mariani, J. (1997). Fear decrease in transgenic mice overexpressing bcl-2 in neurons. *Neuroreport*, 8, 2429–2432.
- Schieve, L. A., Meikle, S. F., Ferre, C., Peterson, H. B., Jeng, G., & Wilcox, L. S. (2002). Low and very low birth weight in infants conceived with use of assisted reproductive technology. *New England Journal of Medicine*, 346, 731–737.
- Schmitteckert, E. M., Prokop, C. M., & Hedrich, H. J. (1999). DNA detection in hair of transgenic mice—A simple technique minimizing the distress of the animals. *Laboratory Animals*, 33, 385–389.
- Sharpe, R. M., Maguire, S. M., Saunders, P. T. K., Millar, M. R., Russell, L. D., Ganten, D., et al. (1995). Infertility in a transgenic rat due to impairment of cytoplasmic elimination and sperm release from the Sertoli cells. *Biology of Reproduction*, 53, 214–226.
- Shinkai, Y., Rathbun, G., Lam, K. P., Oltz, E. M., Stewart, V., Mendelsohn, M., et al. (1992). Rag-2 deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell*, 68, 855–867.
- Smeyne, R. J., Klein, R., Schnapp, A., Long, L. K., Bryant, S., Lewin, A., et al. (1994). Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. *Nature*, 36, 246–249.
- Smith, R. S., Roderick, T. H., & Sundberg, J. P. (1994). Microphthalmia and associated abnormalities in inbred black mice. *Laboratory Animal Science*, 44, 551–560.
- Smithies, O. (1993). Animal models of human genetic diseases. *Trends in Genetics*, 9, 112–116.
- Snouwaert, J. N., Brigman, K. K., Latour, A. M., Malouf, N., Boucher, R., Smithies, O., et al. (1992). An animal model for cystic fibrosis made by gene targeting. *Science*, 257, 1083–1088.
- Steele, E. C., Wang, J. H., Lo, W. K., Saperstein, D., Li, X. L., & Church, R. L. (2000). Lim2(To3) transgenic mice establish a causative relationship between the mutation identified in the Lim2 gene and cataractogenesis in the To3 mouse mutant. *Molecular Vision*, 6, 85–94.
- Strohle, A., Poettig, M., Barden, N., Holsboer, F., & Montkowski, A. (1998). Age- and stimulus-dependent changes in anxiety-related behaviour of transgenic mice with GR dysfunction. *Neuroreport*, 9, 2099–2102.
- Tabuchi, Y., Ohta, S., Arai, Y., Kawahara, M., Ishibashi, K., Sugiyama, N., et al. (2000). Establishment and characterization of a colonic epithelial cell line MCE301 from transgenic mice harboring temperature-sensitive simian virus 40 large T-antigen gene. *Cell Structure & Function*, 25, 297–307.
- Tanaka, S., Oda, M., Toyoshima, Y., Wakayama, T., Tanaka, M., Yoshida, N., et al. (2001). Placentomegaly in cloned mouse concepti caused by expansion of the spongiotrophoblast layer. *Biology of Reproduction*, 65, 1813–1821.
- Tamashiro, K. L. K., Wakayama, T., Akutsu, H., Yamazaki, Y., Lachey, J. L., Wortman, M. D., et al. (2002). Cloned mice have an obese phenotype not transmitted to their offspring. *Nature Medicine*, 8, 262–267.
- Taurog, J. D., & Hammer, R. E. (1996). Experimental spondyloarthropathy in HLA-B27 transgenic rats. *Clinical Rheumatology*, 15, 22–27.

- Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P. C., et al. (1999). Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nature Genetics*, *23*, 99–103.
- Tumminia, S. J., Clark, J. I., Richiert, D. M., Mitton, K. P., Duglas-Tabor, Y., Kowalak, J. A., et al. (2001). Three distinct stages of lens opacification in transgenic mice expressing the HIV-1 protease. *Experimental Eye Research*, *72*, 115–121.
- van der Meer, M., Baumans, V., Olivier, B., & van Zutphen, B. L. M. (2001). Impact of transgenic procedures on behavioral and physiological responses in postweaning mice. *Physiology and Behaviour*, *73*, 133–143.
- van der Meer, M., Costa, P., Baumans, V., Olivier, B., & van Zutphen, B. (1999). Welfare assessment of transgenic animals: Behavioural responses and morphological development of newborn mice. *ATLA-Alternatives to Laboratory Animals*, *27*(Suppl. 1), 857–868.
- van der Meer, M., Rolls, A., Baumans, V., Olivier, B., & van Zutphen, L. F. M. (2001). Use of score sheets for welfare assessment of transgenic mice. *Laboratory Animals*, *35*, 379–389.
- van der Meer, M., & van Zutphen, L. F. M. (1997). Use of transgenic animals and welfare implications. In L. F. M. van Zutphen & M. van der Meer (Eds.), *Welfare aspects of transgenic animals* (pp. 78–89). Berlin: Springer.
- Voigt, J. P., Rex, A., Bader, M., & Fink, H. (2000). From genotype to phenotype—Behavior of the transgenic rat TGR(mRen2)27 as an example. *Reviews in the Neurosciences*, *11*, 37–45.
- Wakayama, T., Perry, A. C. F., Zuccotti, M., Johnson, K. R., & Yanagimachi, R. (1998). Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*, *394*, 369–374.
- Watanabe, M., Ueno, Y., Yamazaki, M., & Hibi, T. (1999). Mucosal IL-7-mediated immune responses in chronic colitis-IL-7 transgenic mouse model. *Immunological Research*, *20*, 251–259.
- Wei, F., Wang, G. D., Kerchner, G. A., Kim, S. J., Xu, H. M., Chen, Z. F., et al. (2001). Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. *Nature Neuroscience*, *4*, 164–169.
- Weiss, S. M., Lightowler, S., Stanhope, K. J., Kennett, G. A., & Dourish, C. T. (2000). Measurement of anxiety in transgenic mice. *Reviews in the Neurosciences*, *11*, 59–74.
- Wolf, E., & Wanke, R. (1997). Growth hormone overproduction in transgenic mice: Phenotypic alterations and deduced animal models. In L. F. M. van Zutphen & M. van der Meer (Eds.), *Welfare aspects of transgenic animals* (pp. 26–47). Berlin: Springer.
- Young, L. E., Sinclair, K. D., & Wilmut, I. (1998). Large offspring syndrome in cattle and sheep. *Reviews of Reproduction*, *3*, 155–163.
- Zahm, J. M., Gaillard, D., Dupuit, F., Hinrasky, J., Porteous, D., Dorin, J. R., et al. (1997). Early alterations in airway mucociliary clearance and inflammation of the lamina propria in CF mice. *American Journal of Physiology & Cell Physiology*, *41*, C853–C859.