

Welfare evaluation of genetically modified mice - An inventory study of reports to the Danish Animal Experiments Inspectorate.

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Summary

The present paper is an inventory study of the reports from 1998 regarding genetically modified animals to The Animal Experiments Inspectorate in Denmark.

The results focus on three areas: percentage of strains experiencing discomfort, percentage of increased mortality, disease incidence and susceptibility to disease, and percentage for which special care was provided. 36% of the strains were reported as experiencing discomfort with 21% experiencing minor discomfort and 15% severe discomfort. In addition 30% of the strains were reported to suffer increases in mortality, disease incidence and susceptibility to disease. The most frequently mentioned conditions being increased mortality, decreased fertility and diabetes. Special care with regard to animal welfare was provided in 34% of the cases. Euthanasia as a humane endpoint, protection against infection and heterozygous breeding were the most frequently mentioned actions.

Systematic information about the welfare of genetically modified animals is limited and the need for this through proper characterization is discussed.

The Danish form from The Animal Experiments Inspectorate is discussed and compared to other

welfare evaluation forms/protocols and improvements are suggested.

Sammendrag

Artiklen indeholder en opgørelse af afrapporteringsskemaer til Dyreforsøgstilsynet vedrørende anvendelse af genetisk modificerede dyr i 1998.

Der fokuseres på tre områder: Procentdel af stammer, der er udsat for belastning som følge af den ændrede genfunktion, procent øget dødelighed, sygdomsforekomst eller nedsat resistens og procent stammer, hvor der iværksættes særlige foranstaltninger med henblik på dyrevelfærd. 36% var belastede som følge af den ændrede genfunktion heraf 21% med en lille belastning og 15% med en svær belastning, 30% led af øget dødelighed, sygdomsforekomst eller nedsat resistens. Øget dødelighed, nedsat fertilitet og diabetes var de oftest nævnte tilstande. Særlige foranstaltninger med henblik på dyrevelfærd blev foretaget hos 34% af stammerne. Aflivning som "humane endpoint", beskyttelse mod infektion og heterozygot avl var de oftest nævnte tiltag.

Systematisk information om transgene dyrs velfærd er begrænset, og behovet for grundig karakterisering af stammer bliver diskuteret.

Det danske skema fra Dyreforsøgstilsynet bliver

diskuteret og sammenlignet med andre velfærdsevalueringsskemaer og forbedringer bliver foreslået.

Introduction

During the last decade both the number of genetically modified strains and the total number of genetically modified mice used for scientific purposes have increased dramatically. These animals are used within a wide range of disciplines including immunology, toxicology, cancer research and genetics and are also used as models of human disease.

The genetic manipulation made in a particular strain might impinge on the welfare of the animal. This may arise both as an expected consequence of the genetic change, as seen in many disease models, and as a non-expected side effect (Mepham *et al.*, 1998). Both severe effects like malformations (McNiesh *et al.*, 1988, Lewis *et al.*, 1993) and milder ones such as reduced fertility are seen (own observations).

It has proved difficult to find information on how many and to what extent genetically modified strains experience reduced welfare. However, more information about their welfare and how to estimate it would enable scientists responsible for genetically modified animals in facilities where genetic manipulations, breeding or experiments are performed to improve their judgements on animal welfare issues.

Details of the genetically modified animals used for experiments in Denmark are described in a form, in questionnaire format, distributed to the licence holders by The Animal Experiments Inspectorate (Dyreforsøgstilsynet) and returned to the Inspectorate on a yearly basis. The material from 1998 has been used in this paper to get an idea of possible welfare disturbances in these animals.

The aim of this inventory was to get a quantitative and qualitative impression of what was reported by researchers on these genetically modified animals and their possible welfare disturbances and to evaluate the method of collecting this information by comparing the Danish form to other similar forms/protocols.

Materials and methods

Evaluation of the information from the Danish forms

Scientists holding a licence to do animal experiments in Denmark must report these activities to The Animal Experiments Inspectorate (Dyreforsøgstilsynet) annually. In case genetically modified animals have been used, they are asked to fill in a special questionnaire-style form entitled "Information about the use of genetically modified animals". Anonymous versions of the forms from 1998 were used for this inventory study. A total number of 80 were registered with the Inspectorate. Two could not be used, one due to a reference that was unavailable and one due to illegible handwriting. Thus, the number included in this study was 78 covering 87 mouse strains.

The form consists of six sections to be filled in. The contents of the form are shown in Table 1. Sections 1, 2 and 3 deal with the identity of the strain, the genetic change and the breeding methods. Sections 4, 5 and 6 deal with the evaluation of the possible discomfort of the animals, their health and the special care taken with regard to animal welfare. The form is filled in by the scientist holding the licence and it is up to him or her to decide how much detail is given. One form can be used for more than one strain.

Sections 4, 5 and 6 were used to evaluate the welfare of the animals. The answers from section 4 (degree of discomfort) were divided into two groups based on whether discomfort was reported or not. The group with reports of discomfort was furthermore divided into two subgroups: one with minor discomfort and one with severe discomfort. This rating was made in accordance with the description made by the licence holders in section 4 of the form. In cases where the description did not address the severity of the condition the author made the rating. Conditions only expected to affect the animal's experience of welfare by a little were rated as minor discomfort and conditions expected to affect the animal's experience of welfare seriously were rated as severe discomfort (Table 2).

This was done in order to achieve a kind of quantification of the conditions affecting the

Table 1. The questionnaire-style form "Information about the use of genetically modified animals" from the Animal Experiments Inspectorate to be filled in

1. Species, type and origin. The name according to the Transgene Animal Data Base should also be stated if possible.
2. Functional change that has been established or aimed at. (To be described, references to relevant literature to be given if possible).
3. Describe how the strain is bred.
4. Evaluation of the discomfort that the animals are exposed to as a consequence of the gene manipulation.
5. Describe an eventual increased mortality, disease incidence or susceptibility to disease in Breeding females, embryos or offspring.
6. State any special care with regard to animal welfare that is provided or is going to be provided.

Table 2. Rating of conditions affecting the welfare of the animals. The rating has been done in accordance with the description made by the licence holders. In cases where the description did not address the severity of the condition the rating has been made by the author.

Conditions rated as Minor discomforts (in alphabetical order)	Conditions rated as Severe discomforts (in alphabetical order)
Aggression	Cystic fibrosis
Decreased fertility	Diabetes
Hypercholesterolaemia	Epileptic seizures
Inhibition of growth	Ileus
Loss of hair	Increased mortality
Lymphoma	Malformation of skull
Osteoporosislike condition	Nephropathy
Ulcers	Rectal prolapse
Weakened immune response	Serious ataxia

welfare of the animal.

Section 5 deals with the possibility of increased mortality, disease incidence or increased susceptibility to disease in breeding females, embryos or offspring. The licence holders are asked to describe these phenomena if present.

The answers to section 5 were registered as a "yes" or a "no" to whether this occurred. In case the answer was "yes" the description given by the licence holder was registered.

Section 6 deals with special care with regard to animal welfare. The licence holders are asked to describe the nature of it if provided. The answers to section 6 were registered as a "yes" or a "no" to the question whether special care was provided. If the answer was "yes", the description given by the licence holder was registered.

On the basis of the replies in section 4, we calculated the percentages of strains experiencing discomfort or no discomfort and also the percentage of animals with little or severe discomfort. On the basis of section 5 we calculated the percentage of strains with increased mortality, disease incidence and susceptibility to disease and on the basis of section 6 we calculated the percentage of strains provided with special care. Sections 4 and 6 were compared to give an impression of the possible relationship between the degree of discomfort and the special care provided.

Comparison of the Danish form to other forms

In order to evaluate the Danish form by comparison to other similar forms/protocols these were retrieved from the literature.

Based on a review of existing schemes, Delpire et al. (1999) suggest a new ethical scheme addressing the use of laboratory animals including genetically modified animals. The data collection in the scheme falls into two parts: the first addressing general issues of justification, scientific relevance, animal suffering and wider social, economic and environmental impacts in relation to laboratory animals. The second part collects data related to generation, production and use of genetically modified animals as well as specific requirements and staff safety measures to be taken.

The German "Gesellschaft für Versuchstierkunde-Society of Laboratory Animal Science" (GV-SOLAS) has given a recommended form for the documentation of genetically modified animals "Dokumentationsblatt für transgene Tiere" (Hedrich et al., 1999). It consists of nine sections dealing with species, name of strain, genetic background, embryo transfer, genetic construct, phenotyping, instructions for breeding/handling and microbiological status.

Relevant parameters of welfare assessment included in these forms/protocols were compared with the Danish form. The extent to which the welfare related parameter had been addressed was rated and divided into four groups: very well, average, inadequately or not addressed (Table 4).

Results

The genetic manipulation was reported to affect the welfare of thirty six percent of the strains, 21% to a minor degree and 15% to a severe degree (Table 3).

In 30% of the strains increased mortality, disease incidence, susceptibility to disease or a combination of these conditions was reported. The most frequent condition described was increased mortality followed by decreased fertility and diabetes. Other conditions like lymphoma, weakened immune response, epileptic seizures, severe ataxia, cystic fibrosis, nephropathy, increased mortality, osteoporosis, inhibition of growth, ileus, rectal prolapses, ulcers and loss of hair were reported.

In 34% of the cases special care was provided. The most frequently reported action was euthanasia as a humane endpoint closely followed by protective measures against infections and applying a heterozygous breeding system instead of a homozygous one. Also special diet, easy access to water, special housing with a reduced stocking density and rejection of the animal as a suitable model for the experiment was reported. Sixty one percent of the strains suffering from a minor degree of discomfort were offered special care, while all strains suffering from a severe degree of discomfort were offered special care in concern of their welfare. A few strains with no discomfort reported were also offered special care.

Table 3. Percentage of strains reported to experience discomfort and a differentiation into severe and minor discomfort.

Strains with discomfort		Strains with no discomfort
36%		64%
Strains with severe discomfort	Strains with minor discomfort	
15%	21%	

N=87

Table 4 is a comparison of the welfare parameters of the Danish form from the Animal Experiments Inspectorate, the GV-SOLAS recommendation and the Ethical Scheme developed by Delpire et al.

The table shows that the Danish as well as the GV-SOLAS form only provide very scant information. Within the ten welfare related categories given, both the Danish and the GV-SOLAS form fail to address several important categories, i.e. number and fate of animals, availability of data and longitudinal observations. Most of the categories are rated "Inadequately addressed" and none of them are rated above "Average".

Thus due to lack of information these two forms provide an inadequate assessment of welfare.

The Ethical Scheme developed by Delpire et al. is given the highest rating "Very well" in all categories except for breeding procedures that receives an "Inadequately addressed". The assessment is that this protocol is capable of producing a much more valid picture of the phenotype than the Danish one.

Discussion

When interpreting the welfare results it should be kept in mind that the raw data were from forms filled in by different people for another purpose than this inventory study. It was, however, possible to gather information of interest from these forms.

Like other authors in this field (*van der Meer & van Zutphen, 1995, van der Meer et al., 1996, Mephram et al., 1998*) we found, that adequate information on the welfare of genetically modified animals was difficult to obtain. Van der Meer et al. (1999, 2001a, 2001b, 2001c) tested the influence of the techniques used for transgenesis on the welfare of the animals showing a 10% perinatal mortality, increased bodyweight and the occurrence of 8% hermaphrodites in gene targeted mice. However, the conclusion was that the procedures per se did not include significant discomfort for the offspring.

To our knowledge no welfare survey of a large number of genetically modified strains has been published. It is therefore difficult to compare the results of this inventory study with similar results. However, 36% of the strains in this inventory were reported to experience discomfort due to their genetic change. This is a surprisingly high percentage.

Differentiation into groups of minor and severe degree of discomfort showed that whereas the majority of these animals suffered only minor discomforts, 15% experienced severe and often fatal conditions. Some of the conditions were expected consequences of the genetic manipulation, as in a cystic fibrosis mouse, while other were unexpected side effects, e.g. a mouse for cancer research was unable to eat normally due to malformations of the skull. However, the form

Table 4. A comparison of the contents of the Danish form from The Animal Experiments Inspectorate(DFT), the GV-SOLAS recommendation of documentation of transgenic laboratory animals and the Ethical Scheme developed by Delpire et al.

The extent to which the welfare related parameter was addressed has been divided into four groups: ***very well, **average, *inadequately and no star = not addressed.

Welfare related parameter	Danish DFT form	GV-SOLAS Form	Ethical Scheme by Delpire et al.
Method of generation of strain - Pronuclear injection/ES cell	*	**	***
Number and fate of animals - Homo-/heterozygous/wildtype - Surplus animals			***
Reason for choosing genetically modified animals - Advantages over non-gen.modified - Scientific validation of model - Contribution to three R's		*	***
Supervision/instruction - Person responsible - Frequency - Certificate of instructions	*	**	***
Breeding procedures - Homo-/heterozygous - Characterization of genetic background	*	*	*
Availability of data - Was existing data retrieved - Will obtained data become available			***
Description of observed phenotype - Behaviour/appearance/performance - Morphology - Pathology - Biochemistry - Mortality/morbidity - Growth/weight - Food/water consumption - Reproduction/fetal development	*	*	***
Longitudinal observations - Changes in pheno-/genotype over time			***
Welfare assessment (evaluation of observations) - Practice followed - Quantification	*	*	***
Special measures concerning welfare - Special housing - Special care - Backcross to strain with fewer problems - Cryopreservation - Humane endpoints established - Pilotstudies	**	*	***

does not enable us to clearly distinguish between discomforts that arise as expected consequences of the genetic change and as unexpected side effects. Such information would be necessary when assessing the benefit of using a particular animal versus the suffering that it is exposed to.

Those 30% of strains reported to have increased mortality, disease incidence or increased susceptibility to disease corresponded well to the fraction of 36% that the licence holders reported as experiencing discomfort, indicating that most of the conditions reported in section 5 of the form had a negative effect on the welfare of the animals. An Italian screening program shows that five out of ten newly generated genetically modified mice have developmental defects (*Costa, 1995*). This is even more than the roughly one third of affected animals, that we have found in this survey; however, the total number of strains in the screening program was limited.

At the University of Washington a surveillance program of ongoing studies has identified both transgenic and knockout lines with unexpected outcomes including increased tumour incidence, diabetes, allergic encephalomyelitis, hydrocephalus, epilepsy, osteoporosis, anasarca and many others (*Van Hoosier, 1999*). These outcomes corresponded well to the findings of this investigation.

The results of this inventory study gave the over all impression that a significant percentage of the genetically modified strains used for experiments in Denmark was affected with regard to welfare a conclusion also reached in other countries (*van der Meer et al., 1996*). In addition approximately one third of the genetically modified strains were reported to receive special care. It seemed as if many conditions could be dealt with in a way that would benefit the welfare of the animal. The necessity of special care in cases where genetically modified animals experience difficulties in coping with the environment has been addressed previously in the literature (*Costa, 1995*).

Euthanasia as the humane endpoint was the most frequent action mentioned under special care indicating that some genetically modified strains suffered severely due to their genetic manipulation.

The investigation showed a good association between the degree of discomfort and special care provided indicating that the users were focusing on the problems that the animals experienced. Strangely, seven strains that had been reported experiencing no discomfort were offered special care anyway. In some cases this could be explained by the fact, that homozygous offspring with symptoms were euthanized as soon as possible (humane endpoint) and that only the heterozygous animals without symptoms were used. However, four of these cases could not be explained on the basis of the information given.

Several licence holders report that heterozygous animals showed no symptoms but that homozygous animals had problems. Based on similar observations, Mephram et al. (1998) recommend heterozygous breeding whenever possible when suffering is seen in homozygous animals.

Other ways of reducing the discomfort of strains showing welfare problems are cryopreservation to eliminate breeding of generations that are not used and backcrossing the transgene to a another background strain, which may give less problems for the animal (*Mephram et al., 1998, van Hoosier, 1999*).

These results add to the widely recognised need for more information about the welfare of genetically modified animals (*van der Meer & van Zutphen, 1995, van der Meer et al., 1996, Mephram et al., 1998, Broom, 1995*). To obtain this, several suggestions for characterization of such animals have been developed. Parameters like appearance, food and water intake, behaviour, clinical signs, weight gain, mortality, fertility, necropsy, histology, clinical chemistry, onset of hair growth, ear and eye opening, stomach filling, neurobehavioral development and fluctuating asymmetry are suggested for this purpose (*Costa, 1995, van Hoosier, 1999, van der Meer et al., 1999, Mertens & Rüllicke, 1999, Stub et al., 2001, Delpire et al., 1999*). Registration systems for genetically modified strains all aim at being both effective and easy to work with and the forms vary markedly as to the parameters in focus and the extent to which they go into details.

The Danish form is limited to three questions (nos.

4, 5 & 6: see Table 1) asking the licence holder to assess discomfort deriving from the altered gene, describe increased mortality, disease incidence and welfare measures taken. The questions are open and often have several statements to address at the same time. This allows the person answering the form to interpret the question in his/her own way. Thus the answers become very different with no possibility of quantification. This makes the forms very difficult to evaluate subsequently as comparison between strains or over time is not possible. Closed questions with a limited number of answers available and quantification into categories is recommended whenever possible. The form should include a much wider range of welfare related parameters and the extent to which they are addressed should be improved.

Conclusion

Having shown that approximately one third of genetically modified animals used for experiments in Denmark is affected in their welfare, we conclude that better registration and control of this area is needed. Therefore, a revision of the Danish form in line with the suggestions from Delpire et al. is recommended. In combination with the comprehensive neuro-behavioral assessment "SHIRPA" described by Rogers et al., (1997) an appropriate protocol could be developed.

This would on the one hand enable a thorough assessment of the justification of generating and using a genetically modified animal, and on the other hand also address animal welfare issues more systematically. It is furthermore recommended that the forms are developed to make sure that questions as well as answers are less ambiguous – e.g. by more carefully specifying the character of the demanded information and operating with distinct categories.

The Danish form however, has the advantage of being intended as a follow-up on experiments already given permission and performed. This gives the licence holder the opportunity to report side effects unknown to him before the experiment.

The ideal situation for the authorities controlling the welfare of genetically modified animals used in experiments would be a comprehensive scheme

included in the application for using the animal in an experiment followed up by a short feed-back afterwards. This inventory study covered animals used for experiments only. According to Danish legislation, generating a genetically modified animal and using one for scientific purposes is regarded as an experiment and must therefore be reported to the Animal Experiments Inspectorate. Animals bred for maintenance only are not necessarily registered. Better legislation and thorough characterization of every genetically modified strain in the future would give a much better background for evaluating the welfare aspects of these animals.

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