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Genetically modified animals in research: an analysis of applications submitted to ethics committees on animal experimentation in Sweden

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Abstract

The use of genetically modified (GM) animals in biomedical research has increased during recent years and its ethical aspects have been subject to ongoing academic discussion. In order to reinforce this discussion, we analysed applications submitted to animal ethics committees in Sweden during 2002. The aim was to investigate the researchers' statements concerning the production and use of GM animals, as well as the committees' assessments of the applications. For our analysis, we constructed an analytic form. In part, we included the questions and categories of the mandatory application form, noting for example species, degree of severity regarding pain and distress, the management of pain, and endpoints. In addition, we included our own specific questions and categories, and classified the applications accordingly. In particular we focused on the methods of GM animal production and on the expected clinical symptoms attributable to genetic modification and experimental use. Our analysis, which was partly quantitative and partly qualitative, revealed that applications were often approved by the committees despite containing insufficient information regarding ethically relevant aspects, that the arguments for using GM animals were often unclear, and that some applicants indicated awareness of possible unintentional welfare effects attributable to genetic modification. In more than 36% of the applications, obvious or minor clinical symptoms attibutable to genetic modification were expected. However, we also noted that many applicants emphasised that certain GM animals were to be used without the expectation that the animals would display any clinical symptoms. This was obviously viewed as an ethical advantage.

Keywords: *animal ethics, animal experimentation, animal welfare, committees, genetically modified animals, transgenic animals*

Introduction

Genetically modified (GM) animals are becoming increasingly important in research (Stokstad 1999) and several expert reports and policy declarations have discussed their production and use (eg CCAC 1997; van Zutphen & van der Meer 1997; Mepham *et al* 1998; Royal Society 2001; BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement 2003). However, to our knowledge, no ethical analysis has yet been conducted of applications submitted to animal ethics committees, or other legal bodies, regarding the production and use of GM animals. With this in mind, we carried out an empirical study in Sweden on how research involving the production and use of GM animals was assessed by the official animal experimentation review system. Sweden is particularly interesting in this regard as it has had animal ethics committees, with large lay representation, since 1979, and because since 1998 the decisions of these committees are not only advisory but also regulatory.

The purpose of this study was to analyse applications from the year 2002 submitted to the seven ethics committees on animal experimentation in Sweden regarding the production and use of GM animals. There were two major objectives for carrying out the study. First, we wanted to reinforce the academic discussion on ethical aspects of the production and use of GM animals by providing empirical knowledge about (a) how researchers present the requested information in the applications they submit to the committees and (b) how the animal ethics committees assess the applications. Second, we wanted to improve the basis for future assessment by (a) clarifying the ethical issues and (b) suggesting additional sections to the application form.

The year 2002 was chosen because we wanted to study a year as recent as possible — particularly important in a research field with rapid development of technologies — and because a new mandatory application form was introduced and used from this year onward.

In Sweden there are seven committees, situated at courts and linked to the major universities. Each committee consists of 12 members (and deputies), with membership evenly divided between researchers and animal house personnel on the one hand, and laypersons including representatives from political parties and animal protection organisations on the other. In addition, there is a chairperson (and a deputy) with judicial experience (Animal Welfare Ordinance 1988, Sections 43–44). There are no academics specialising in ethics on the committees. During the study period, the overall responsibility for the review system lay

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with the Swedish National Board for Laboratory Animals ('Centrala försöksdjursnämnden'); however, in 2004 the Board was replaced with the Swedish Animal Welfare Agency ('Djurskyddsmyndigheten').

It is mandatory, for each animal experiment, that the principal investigator completes an application form. The information provided by the applicant constitutes the basis for the assessment made by the committee, which may request supplementary information and require modifications to the experiment before approval is granted. The same application form is used by all the committees and for both non-GM and GM experiments. The protocols of the committees do not include any reasons for approval, only for rejection.

The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS number 123, 1986) and the European Union Council Directive regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC), together with the Swedish Animal Welfare Act (1988) and Animal Welfare Ordinance (1988), govern the work of the Swedish committees. However, it should be noted that there is a difference between the EU regulation and the Swedish legislation with regard to the definition of 'animal experiment'. The former only includes the use of animals for scientific purposes that may involve animal suffering, whereas the latter also includes the use of animals for scientific purposes that do not involve any such suffering.

In the Animal Welfare Act, it is stated that "Animals shall be treated well and shall be protected from unnecessary suffering and disease" (1988, Section 2 [1]) and that "Animals... shall be accommodated and handled in an environment that is appropriate for animals and in such a way as to promote their health and permit natural behaviour" (1988, Section 4 [1]). The Animal Welfare Ordinance states that "When considering specific cases the committee shall weigh the importance of the experiment against the suffering inflicted on the animal" (1988, Section 49 [1]). It should be emphasised that during our analysis of the applications we remained within this legal and ethical framework.

It is not completely obvious how to characterise this framework more precisely in ethical terms. Its key feature is the balancing of human benefit and animal harm, which together with its primary focus on animal pain and suffering, suggest a utilitarian platform. However, the concern for natural behaviour indicates that, although primarily so, the framework is not exclusively utilitarian.

The implicit concept of animal welfare also deserves a comment. It seems that in this legislative framework there are traces of all three fundamental animal welfare concerns proposed by Professor David Fraser: feeling; functioning; and natural living (see eg Fraser 1993, 2003; Fraser *et al* 1997). The primary focus on 'suffering' shows a concern for animal feeling (compare Dawkins 1980; Duncan 1993); the focus on 'disease' and 'health' indicates a concern for biological functioning (compare Broom & Johnson 1993; Broom 1996); and the focus on 'natural behaviour' suggests a concern for natural living (compare Kiley-Worthington 1989; Rollin 1993).

On the application form, the applicant is requested to clarify whether the application concerns the production of GM animals, their use, or both their production and use (Centrala försöksdjursnämnden 2002, pp 43–47). The guidelines for the application form define GM animals as "animals, the genomes of which have been modified by technical methods, for example transgenic animals or knock-out animals" (our translation; Centrala försöksdjursnämnden 2002, p 43). This definition stresses that the technology used determines what shall be considered as a GM animal, thereby excluding conventional breeding and spontaneous mutations. This is in line with the terminology in the European Union Council Directive 90/220/EEC, which states that a 'genetically modified organism' is an "organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination". Moreover, this understanding conforms to the definitions used in academic investigations. For example, the 2001 Report from the Royal Society defines the term 'genetically modified animals' as "animals modified either via a technique known as transgenesis (when individual genes from the same or a different species are inserted into another individual) or by the targeting of specific changes in individual genes or chromosomes within a single species — targeted removal of genes (knock-outs) or targeted addition of genes (knock-ins)" (Royal Society 2001, p 3).

As background to our study, we investigated the number of applications submitted during the five-year period 1998–2002 (note that we refer to the number of applications submitted in a particular year as opposed to those decided on, which is done by eg Hagelin *et al* 2003). The total number of applications regarding the production and use of GM animals per year almost doubled during this initial study period, from 186 in 1998 to 332 in 2002. Moreover, the GM portion of the total number of applications to animal ethics committees increased from 11.5% in 1998 (186 of 1622) to 19.8% in 2002 (332 of 1679). These findings may be a reflection of the increased interest among scientists in using GM animals because these animals offer sharper and kinder tools, as suggested by Stokstad (1999). The increased general availability of GM animals to researchers lacking the resources or skills needed to produce such animals themselves is also likely to contribute to the observed trend.

During the five-year period 1998–2002, the total number of approved applications involving the use of GM animals (n = 1024) was much higher than both the total number of applications involving the production of such animals (n = 60), and the total number of applications involving both the production and use of GM animals (n = 207). The higher number of applications regarding use compared with production can probably be partially explained by the fact that it is a laborious and expensive undertaking to produce GM animals. In addition, scientists may obtain GM animals from colleagues, or purchase them, if the animals are appropriate for their intended research.

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Materials and methods

Our research questions were:

• How do researchers present the requested information in applications regarding GM animals submitted to ethics committees on animal experimentation in Sweden?

• How do the committees assess the applications?

In order to answer these questions, we carried out a detailed analysis of applications submitted to the seven animal ethics committees in Sweden during 2002. We analysed applications involving the production of GM animals, the use of GM animals, and both the production and use of GM animals, focusing on a few important aspects clearly related to genetic modification. We made quantitative investigations concerning some of these aspects and qualitative analyses concerning others. We only investigated approved applications (325 applications, out of a total of 332); thereby, we expected to get an indication of which GM animal experiments would actually be carried out, which was of particular ethical interest. In the analysis, we included not only the application forms but also correspondence between the committee and applicant.

On the application form, the applicant — as mentioned above — is requested to clarify (by ticking boxes) whether the proposed application concerns the production of GM animals, the use of GM animals, or both the production and use of GM animals. Moreover, the applicant is requested to classify (also by ticking boxes) the experiment as a whole with regard to the 'degree of severity' of animal pain or other distress. There are three categories: mild, moderate and severe. The guidelines for completing the application form provide a list of examples in each of the respective categories. However, the list is not complete and may require some judgment on behalf of the applicant. The main part of the application form contains nine sections (Centrala försöksdjursnämnden 2002, pp 43–47):

• title

• purpose

• alternative methods

• special requirements for documentation

• species and number

• experimental design

• animal care and keeping

• the situation of the animals and the endpoint of experiment

• methods of anaesthesia and euthanasia.

For the analysis of the applications, we constructed an 'analytic form'. On this form, we included some of the categories found in the application form, namely 'production', 'use' and 'both production and use', and the three 'degrees of severity'. In addition, we created additional categories that related to different parts of the application form. We excluded the section concerning 'special requirements for documentation' because this was not relevant to the purpose of our investigation. We also excluded the issue of the number of animals to be used in the experiment because the applicant is explicitly requested only to state the total number of animals to be used, not the number of GM animals to be produced or used.

The sixteen sections of our analytic form, with some additional information, are outlined below.

(1) *Production and use of GM animals.*

(2) *Research fields*. On the application form, the applicants are requested to provide information on the department to which they belong, but not to define their field of research. This makes it difficult to define fields of research in a way that avoids overlap. Therefore, we were satisfied with a list of fields and did not investigate their relative distribution.

(3) *Purpose*. We focused on direct purposes only, ie what the applicant directly intended to do, independent of possible future applications. We discerned two major direct purposes: to obtain basic biological knowledge and to obtain knowledge related to human disease and health. The latter purpose is directed towards causes of disease or treatment, whereas the former is not. We also included the category 'other' for purposes such as education or for use in an agricultural context.

(4) *Alternatives to animal experimentation.*

(5) *Reasons for producing or using GM animals.* A pilot study indicated that it was not possible to use specific categories such as 'necessary' or 'suitable' on the analytic form because the arguments were unclear. Given this, we used the broad category 'necessary or suitable given the purpose of study'.

(6) *Species.*

(7) *Methods of producing GM animals.* We focused on three types of method: pronuclear microinjection (the classical method used for transferring a gene); the embryonic stem cell method (that offers the possibility of gene targeting including gene inactivation, ie knock-out); and conditional methods (that allow for genetic modifications that are tissue-specific or temporally specific, ie the activity can be turned on and off) (Pinkert 2002; Houdebine 2003).

(8) *Types of genetic modification.* We concentrated on knock-out, insertion of a foreign gene — human or otherwise — producing a new protein, and insertion of a gene leading to overexpression, ie production of a larger amount of a particular protein. We did not distinguish between genetic modifications that were specific (with regard to tissue or time) and those that were general.

(9) *Unintended welfare effects.*

(10) *Degree of severity of the experiment as a whole.* The guidelines for completing the application form do not give any examples of experiments — causing animal pain or distress that are classified as 'mild', 'moderate' or 'severe', respectively — that are specifically related to GM animals.

(11) *Expectancy of clinical symptoms attributable to genetic modification.* A key aspect of GM animal welfare concerns the expectancy of clinical symptoms attributable to genetic modification; another concerns clinical symptoms attributable to experimental use (see next point). Our definition of 'clinical symptoms' refers to detrimental changes to welfare-related appearance, behaviour or function.

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Table 1 The percentage of applications proposing the production, use, and both production and use of GM animals (n = 325).

Animal experiment	Approved applications regarding GM animals (%)
Production	4.3
Use	80.3
Both production and use	15.4

(12) *Expectancy of clinical symptoms attributable to the experimental use of GM animals.*

(13) *Measures for managing animal pain and distress.*

(14) *Endpoints.*

(15) *Decisions of the committees.* We only included applications that were submitted to the committees and on which the committees made a decision the same year or later. Applications that were 'withdrawn' were not included, because no decision was made by the committees.

(16) *Reservations against approval.* No categories were created in advance for these reservations, because this was not possible on the basis of the application form. Instead we used a qualitative approach, recognising certain types of argument in the various reservations.

Results

The results related to the sections on the analytic form were as follows; in a few cases we have included additional information obtained during the research process.

(1) *Production and use.* The applications concerned the production of GM animals, the use of such animals, and both the production and use of GM animals (Table 1).

(2) *Research fields.* Applications were submitted by researchers from a great variety of research fields, for example, genetics, cell biology, molecular biology, physiology, immunology, cancer research, neuroscience, pharmacology, diabetes research, and cardiovascular research.

(3) *Purpose.* We found that 36.6% of the applications had basic biological knowledge as their direct purpose, whereas 60.3% had knowledge related to human disease and health as their direct purpose. Only 3.1% of applications had alternative purposes, such as education. We found no applications with a direct purpose related to production of therapeutic proteins, xenotransplantation, or agricultural purposes, for example improved farm animal health.

(4) *Alternatives to animal experimentation.* All applications stated that the use of a whole animal was necessary given the purpose of the study. At times this point was proposed explicitly and with detailed comments, and at other times only implicitly. Occasionally, only 'no' was stated in response to this question.

(5) *Reasons for the production or use of GM animals.* All justifications were 'necessary or suitable given the purpose of study'. There were often no explicit arguments, only implicit ones.

(6) *Species.* In 99.1% of applications the GM mouse was to be produced and/or used. However, there were a few examples of other species to be produced and/or used, sometimes with the mouse (1.8%); these were rat, zebra fish, and chicken (eggs).

(7) *Methods of producing GM animals.* The most common methods proposed were, in the following order: pronuclear microinjection (43.8%), the embryonic stem cell method (35.9%), and conditional methods (eg the Cre-LoxP method) 28.1%. Occasionally, we found that in a single application, two or even three different methods were proposed. Of all the applications, 7.8% did not provide any information about which method of production was to be used.

A classification problem arose in the case of animal experiments on somatic gene transfer. In the established terminology, GM animals include only those whose genomes have been modified using the germ-line. However, we found eight applications in which the modifications were to be made only using somatic cells: the applicants still viewed the experiments as involving GM animals. They classified the experiments as involving the production of GM animals, the use of GM animals, or both the production and use; the committees did not dispute these classifications.

(8) *Types of genetic modification.* In total, 57.5% of applications proposed 'knock-out' genetic modification. In 24%, a foreign gene was to be inserted producing a new protein. In 11.1%, the genetic modification was intended to lead to overexpression. Occasionally, two or three types of genetic modification were proposed to be used in the same application. In 19.7% of applications, the type of genetic modification proposed was not stated (Table 2).

(9) *Unintended welfare effects.* The possibility of unintended welfare effects was stressed in 40.6% of the applications regarding the production or both production and use. In 3.1% it was stated that unintended welfare effects would probably not occur. In 56.3%, the aspect of unintended effects was not stated.

(10) *Degree of severity of the experiment as a whole.* In total, 43.1% and 52.9% of the applicants viewed the degree of severity of the experiment as a whole as mild or moderate, respectively. The degree of severity was viewed as severe in only 4.0% of applications. In 50% of the applications regarding the production of GM animals, and in 40% of applications regarding both production and use, the degree of severity was viewed as mild (Table 3). We also found a few applications in which the committee had changed the degree of severity from mild to moderate, and one case in which the committee had changed it from moderate to severe.

(11) *Expectancy of clinical symptoms attributable to genetic modification.* In total, 36.3% of applications expected that the animals would exhibit obvious or minor clinical symptoms attributable to genetic modification. In 25.5% no clinical symptoms were to be expected and in 34.2% it was not stated whether the animals were expected to exhibit clinical symptoms. We also found one example of expected improvement of welfare. These animals were to be used

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Table 2 Type of genetic modification proposed. Figures show percentages of approved applications regarding production, use, and production and use of GM animals.

Type of genetic modification	Production (%)	Use (%)	Both production and use (%)	Total (%)
Knock-out*	50.0	58.2	56.0	57.5
Foreign gene inserted*	42.9	19.2	44.0	24.0
Overexpression*	7.1	10.0	18.0	11.1
Not stated	7.1	23.4	4.0	19.7

* sometimes together with other types of genetic modification

Table 3 Degree of severity of the experiment as a whole. Figures show percentages of approved applications regarding production, use, and production and use of GM animals.

Degree of severity	Production (%)	Use (%)	Both production and use (%)	Total (%)
Mild	50	43.3	40	43.1
Moderate	50	52.9	54	52.9
Severe	0	3.8	6	4.0

Table 4 Expectancy of clinical symptoms attributable to genetic modification. Figures show percentages of approved applications regarding production, use, and production and use of GM animals.

Clinical symptoms	Production (%)	Use (%)	Both production and use (%)	Total (%)
Obvious clinical symptoms expected	35.7	16.9	52.0	23.1
Only minor clinical symptoms expected	21.4	13.8	8.0	13.2
No clinical symptoms expected other than in the long term	0	n/a	n/a	n/a
No clinical symptoms expected before experimental use, but can be expected in the long term	n/a	4.6	0	n/a
No clinical symptoms expected	21.4	27.6	16.0	25.5
Improved welfare	0	0.4	0	0.3
Not stated	21.4	36.8	24.0	34.2

Table 5 Expectancy of clinical symptoms attributable to the experimental use of GM animals. Figures show percentages of approved applications.

Clinical symptoms	Use (%)	Both production and use (%)	Total (%)
Clinical symptoms expected	56.3	52.0	55.6
No clinical symptoms expected	32.6	22.0	30.9
Not stated	11.1	26.0	13.5

Table 6 Measures for managing animal pain and distress.

Measures	Approved applications regarding GM animals (%)
Euthanasia*	56.0
Pain relief*	8.3
Enriched environment*	11.7
No measures despite pain or distress	3.4
No measures needed	6.2
Killing before any pain or distress appears	4.9
Not stated	22.8

* sometimes together with other measures

because they had an improved immune system attributable to genetic modification (Table 4).

(12) *Expectancy of clinical symptoms attributable to the experimental use of GM animals.* In total, 55.6% of the applications stated that clinical symptoms were to be expected. In 30.9% of applications it was stated that no clinical symptoms were to be expected, and in 13.5% it was not stated whether any clinical symptoms were to be expected (Table 5).

(13) *Measures for managing animal pain and distress.* The results can be seen in Table 6. Note that 'pain relief' referred to post-surgery and other pain relief, rather than anaesthesia during surgery. 22.8% of applications did not state any measures for managing animal pain and distress.

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Table 7 Approvals and rejections (n = 332).

Decision of committee	Applications regarding GM animals (%)
Approved	97.9
Rejected	2.1

Table 8 Approvals (n = 325).

Decision of committee	Approved applications regarding GM animals (%)
Approved without comment	18.8
Approved after supplementary information	51.7
Approved on certain conditions*	27.4

* sometimes after supplementary information only

(14) *Endpoints.* 2.2% of applications did not state any endpoint, although the guidelines state that there should always be an endpoint described.

(15) *Decisions of the committees.* 2.1% of the applications were rejected (Table 7). 51.7% of the applications were required to provide supplementary information before being approved. 27.4% were approved only on certain conditions, and sometimes only after supplementary information was provided (Table 8). These conditions may concern, for example, the method of killing or method of injection.

(16) *Reservations against approval.* There were 79 reservations listed by the committees against approval of applications, for example:

• the experiment implies unacceptable animal suffering compared with the expected result;

• a proper balancing of suffering and benefit has not been performed;

• independent of suffering, the purpose is not important enough;

• the study will most probably yield neither relevant nor new results;

• the phenotype is unpredictable;

• relevant information is lacking and has not been requested by the committee;

• the handling of the application by the committee has not been acceptable.

Discussion

General comments

This study found that only 2.1% of applications submitted during 2002 regarding the production and/or use of GM animals were rejected. This result is in line with the general trend in Sweden; Hagelin *et al* (2003) found that only 6.2% of applications from 1989–2000 were postponed, rejected or withdrawn. The low percentage of applications that are rejected may be explained, in part, by the mandatory pre-review of each application at the applicant's department before it is submitted to the committees. Moreover, the committees — as mentioned previously — may require modifications and approve an application only under certain conditions.

Another important finding of this study was that many approved applications did not provide all the information they were obliged to according to governmental guidelines. This was also indicated in some of the reservations listed by the committees against approval of some applications. We found that information was lacking in several central issues; for example, the method of production, the type of genetic modification, the possibility of unintended welfare effects, whether animals would exhibit clinical symptoms attributable to genetic modification, whether animals would exhibit clinical symptoms attributable to experimental use, measures for managing animal pain or distress, and a clear endpoint to the experiments. The committees approved the applications despite this lack of information. However, we have an indication — resulting from informal discussions with people involved with the committees — that sometimes there may be unrecorded oral communication between members of the committees and the applicants. Therefore, it is possible that some additional information was provided in this way. The extent of this is impossible for us to assess, as we have only studied written material. However, it is reasonable to expect the animal ethics committees to conform to the common administrative policy of public accountability and openness. The committees are part of the legal system and their decisions are not only advisory but also regulatory. It is not satisfactory for there to be a common practice of not recording all relevant information. Furthermore, it must be possible for society to scrutinise the decisions made by the committees, and this is only possible if all relevant information is recorded and accessible.

Comments on certain aspects of genetic modification

The purpose of GM animal experiments

We found that 37% of the applications had basic biological knowledge as their direct purpose, whereas almost 60% had health-oriented knowledge as their direct purpose. From an ethical point of view, the purpose of study is of vital importance as it restricts the range of possible methods that can be used, ie GM methods or non-GM methods, and is closely related to the expected human benefit. Public surveys indicate that many people assign more weight to health-oriented research than to basic biological research (see eg Aldhous *et al* 1999). However, in the history of science many medical advances would never have been discovered in the absence of basic research with no foreseeable direct application. Therefore, given the ethical framework of the Swedish animal welfare legislation, at least some basic research involving animal harm may be justifiable. But it is difficult to know where to draw the line. Some would argue that all or nearly all human knowledge interests carry enough weight, others that severe — or even moderate — animal harm may outweigh certain basic knowledge interests and sometimes even certain health-oriented ones. In some reservations to

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the approvals, we saw examples of more restricted views on what purposes carry sufficient weight.

The likelihood of a beneficial outcome of an experiment involving GM animals is also very important in the ethical assessment; however, this information is not explicitly requested on the application form. Furthermore, what could 'likelihood' mean in this context? The best way to interpret this is in terms of the expected human benefit. We can hardly know anything about 'objective probabilities' regarding future applications of scientific knowledge, whatever 'likelihood' may mean. It seems better to understand 'likelihood' in terms of a subjective degree of trust or expectancy. If the likelihood of human benefit of a particular animal experiment is low, perhaps it is not sufficient to justify the experiment. However, if the likelihood of human benefit is high, it may contribute to the justification of the experiment. Assessing the likelihood of benefit of a particular experiment is certainly very difficult, for applicants as well as committees. However, commonly the likelihood — in a subjective sense — of producing medical benefit is higher for health-oriented experiments than for basic ones. The reason for this is that they are aimed directly at contributing to solutions to health problems and are closer in time to such solutions than are basic experiments.

In the long run, it is not satisfactory for the assessments of the committees to be based on subjective expectancies. Therefore, a feedback system should be developed for investigating the success of specific research programs which involve many individual experiments. In this way, the assessments of the committee may be carried out in a more impartial way.

Reasons for producing and using GM animals

The justifications for using GM methods fell into the broad category 'necessary or suitable given the purpose of study'. Consequently, it was often difficult to assess whether GM animals were considered necessary or only suitable: the arguments were often unclear. However, from an ethical point of view, it is very important to make a clear distinction, not least in order to make a proper ethical evaluation of the experiment. Moreover, it might be important to distinguish two different senses of 'suitable'. 'Suitable' may mean 'scientifically better than using other GM or non-GM methods' or 'scientifically as good as using other GM or non-GM methods'.

With this in mind, researchers should clarify which of the following three arguments they wish to make:

• Given the purpose P, it is scientifically necessary to produce (or use) the GM animal A.

• Given the purpose P, it is scientifically better to produce (or use) the GM animal A than to use other GM methods or non-GM methods.

• Given the purpose P, it is scientifically as good to produce (or use) the GM animal A as to use other GM methods or non-GM methods.

'The purpose P' is a particular purpose, for instance, to obtain a particular piece of basic biological knowledge or a particular piece of health-oriented knowledge. 'The GM animal A' is an animal (or a number of individual animals) to be produced or already produced by a particular method of genetic modification.

Two stages of justification are required. First, the purpose must be justified. A good reason must be provided for believing that the purpose of obtaining a particular piece of basic knowledge or a particular piece of health-oriented knowledge would be beneficial to society. Second, the scientific necessity or suitability must be justified. A good reason must be given for believing that it is 'scientifically necessary', 'better' or 'as good' to produce or use this particular GM animal A in order to realise this particular purpose.

Three examples focusing on the distinction between GM and non-GM methods are as follows. (1) The aim is to discover the function of a particular gene. In this case, the applicant could argue that it would be scientifically necessary to make a knock-out; no non-GM methods are possible. (2) The aim is to create an animal model of a particular disease, which is also possible using non-GM methods. In this case, the applicant wants to show that it would be scientifically better to make a GM model than to induce the disease — eg diabetes — using non-GM methods. (3) The aim is to create an animal model of a particular disease, which is also possible using non-GM methods, but in this case the GM disease model is as good as the non-GM model.

It should be pointed out that the Swedish animal welfare legislation does not suggest that non-GM methods are preferable to GM methods. It is the balance of human benefit and animal harm that is important, and animal harm is primarily viewed in terms of pain and suffering.

Welfare of GM animals

The welfare of GM animals can be divided into two categories. The first concerns the welfare of animals used in the process of production. The second concerns the welfare of the resulting GM animals, which are to be used in further studies. Regarding both aspects, it is important to distinguish between intentional suffering attributable to, for example, gene knock-outs with known or unknown effects or the use of GM animals as disease models, and unintentional suffering attributable to, for example, random integration or mutagenesis.

Fifty percent of the applications regarding the production of GM animals, and 40% of those regarding both production and use, classified the degree of severity regarding animal pain and distress as 'mild'. However, it could be argued that, according to the guidelines, this classification should be at least 'moderate'. Certainly, the guidelines for the completion of the application form include no explicit directive regarding the classification of the production of GM animals, and many of the steps of production belong to the category 'mild', for example hormone stimulation, killing of donor females, vasectomy of males with anaesthesia, tail

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biopsy, and ear marking. However, the surgical implantation of genetically modified embryos into pseudo-pregnant females with anaesthesia reasonably belongs to the category 'moderate'. This classification fits the second example of 'moderate' degree of severity given in the guidelines, namely "major surgical interventions with anaesthesia in stomach cavity, chest cavity..." (our translation; Centrala försöksdjursnämnden 2002, p 46).

Regarding the expected welfare of the resulting GM animals, we classified the applications on the basis of their expected clinical symptoms. We found that information was lacking surprisingly often and was not requested by the committee. It is hard to say whether this was because of unawareness of ethical implications or the lack of interest in the broader effects on the phenotype, or if these effects were assumed to be commonly known. In any case, a lack of information is not ethically acceptable.

We also found that obvious or minor clinical symptoms attributable to genetic modification were expected in 36% of the applications. It is interesting to compare this finding with the result of an inventory study of reports to the Danish Animal Experiments Inspectorate. In this study, Thon *et al* (2002) found that 36% of the genetically modified strains of animals were reported as experiencing discomfort. Now, clinical symptoms and experienced discomfort are different things, so are estimations before experiments and reports afterwards, as well as a focus on the number of applications and on animal strains. This means that a direct comparison between this study and the study by Thon *et al* (2002) is not possible. Nevertheless, both studies point in the same direction, namely that a substantial portion of GM animals may have rather poor welfare.

If we turn to the positive results regarding the welfare of GM animals, it is worth emphasising that only 4% of the applications expected the degree of severity of the experiment as a whole to be severe. Moreover, in 25% of applications, it was stressed that no clinical symptoms attributable to genetic modification were expected. This was probably emphasised as this is an ethical advantage; we agree. It would indeed be an advantage from the perspective of animal welfare if animals could be used without manifesting any clinical symptoms, or only minor symptoms, or even symptoms only in the long run. In one application even improved animal welfare was expected.

It is also important to note that, from an animal welfare perspective, GM methods might sometimes be better than non-GM methods. For example, genetically engineered diabetes models may be more favourable than pharmacologically or surgically induced models. Let us particularly point out an ethical advantage of conditional methods. Tissue-specific and temporally specific modifications provide a mechanism for minimising negative effects on animal welfare (BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement 2003, p 6); therefore, the fairly common use of these methods, found in the applications, is positive.

With regard to unintentional animal suffering, the method of production is also relevant. Such welfare effects primarily occur when pronuclear microinjection is used, which is characterised by random integration. In the embryonic stem cell method, random integration is not an issue, because this method involves homologous recombination carried out *in vitro*; in principle, only embryonic stem cells with the desired genetic modification are injected into the blastocyst, whereas those with insertional mutations are not. Because of this, unintended effects of using this type of method are rare in living animals (Buehr *et al* 2003). Therefore, it appears that the embryonic stem cell method has an ethical advantage with regard to unintentional animal welfare effects compared with pronuclear microinjection.

The possibility of unintended and unpredictable welfare effects was emphasised in 40% of the applications regarding the production or both the production and use of GM animals; however, in 56% of the applications this was not mentioned. To some extent, this may be attributable to the use of the embryonic stem cell method rather than pronuclear microinjection, although unintentional welfare effects may still occur from the embryonic stem cell method. Even if a gene is correctly inserted, the animal carrying it may exhibit an unexpected phenotype (Buehr *et al* 2003). However, it is important to emphasise that there are unpredictable welfare effects in all animal experimentation and that GM methods may actually often make the outcome more predictable, for example, when compared with selective breeding.

Finally, when using already produced GM animals, the problems of clinical symptoms are quite different. The researchers may be informed by colleagues from whom they obtain the animals or by the company from which the animals are bought. However, our analysis indicates that there may be difficulties in detecting clinical symptoms, and more research is certainly needed. A practical and rather promising method is welfare scoring (Crawley 2000; van der Meer *et al* 2001; Jegstrup *et al* 2003).

Balancing human benefit and GM animal harm

As emphasised in the introduction, the main feature of the ethical framework in the Swedish animal welfare legislation is the balancing of human benefit and animal harm, primarily conceived in terms of animal pain and suffering. Whether or not the committees balanced the expected human benefit and the expected animal harm in particular GM experiments in an ethically acceptable way is, of course, a key question. There is certainly no consensus regarding case-by-case balancing, and sometimes the balancing carried out by the committees was objected to in the reservations listed against approval of some applications. It is clearly beyond the scope of this paper to perform a harm–benefit analysis of particular applications — or even a careful selection of them. However, in principle, society accepts animal experimentation involving the production and use of GM animals, if it can be expected to lead to significant advances in understanding basic biological processes and to provide major medical benefit.

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Proposal: supplements to the application form

On the basis of this investigation, we suggest that the application form, and the guidelines for its completion, be supplemented in order to facilitate the assessment of experiments involving GM animals. Applicants should be explicitly required to specify which GM methods are to be used and to explain whether these methods are scientifically necessary, better or only as good as non-GM methods. Moreover, they should be explicitly required to clarify expected clinical symptoms attributable to genetic modification and the possibility of unintended and unexpected welfare effects. There should also be an explicit recommendation that the degree of severity — with regard to animal pain and distress — of the production of GM animals be classified as at least 'moderate'.

Animal welfare implications

We conclude that the applications regarding GM animals submitted to Swedish ethics committees on animal experimentation in 2002 were often approved despite lacking important information regarding animal welfare. We found that obvious or minor clinical symptoms attributable to genetic modification were expected in 36% of the applications. We also noted that many applicants stressed that certain GM animals were to be used without manifesting any clinical symptoms. This was obviously considered an ethical advantage. Some applicants indicated an awareness of the possibility of unintended welfare effects attributable to genetic modification, whereas some did not. Only very few applications classified the degree of severity of the experiment as a whole — as regards animal pain and distress — as severe. If this classification reflects the actual welfare of GM animals, it is positive.

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References

Aldhous P, Coghlan A and Copley J 1999 Let the people speak. *New Scientist 162*: 26-31

Animal Welfare Act ('Djurskyddslagen') 1988: 534 (with later revisions and supplements), In: Centrala försöksdjursnämnden ('The Swedish National Board for Laboratory Animals') 1999 *Provisions and general recommendations relating to the use of animals for scientific purposes*. Stockholm, Sweden

Animal Welfare Ordinance ('Djurskyddsförordningen') 1988: 539 534 (with later revisions and supplements), In: Centrala försöksdjursnämnden ('The Swedish National Board for Laboratory Animals') 1999 *Provisions and general recommendations relating to the use of animals for scientific purposes*. Stockholm, Sweden

Broom D 1996 Animal welfare defined in terms of attempts to cope with the environment. *Acta Agriculturae Scandinavica, Section A — Animal Science (Suppl 27)*: 22-28

Broom DM and Johnson KG 1993 *Stress and Animal Welfare*. Chapman & Hall: London, UK

Buehr M, Hjort JP, Hansen AK and Sandøe P 2003 Genetically modified laboratory animals — what welfare problems do they face? *Journal of Applied Animal Welfare Science 6(4)*: 319-338

BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement 2003 Refinement and reduction in production of genetically modified mice. *Laboratory Animals 37 (Suppl 1)*: S1-S49

Canadian Council on Animal Care (CCAC) 1997 *The CCAC Guidelines on Transgenic Animals*. CCAC: Canada

Centrala försöksdjursnämnden ('The Swedish National Board for Laboratory Animals') 2002 *Författningar, allmänna råd och anvisningar om användningen av djur för vetenskapliga ändamål*. CFN:s skriftserie Nr 45, Stockholm, Sweden [Title translation: Statutory regulations, general recommendations and guidelines regarding the use of animals for scientific purposes]

Crawley JN 2000 *What is Wrong with my Mouse? Behavioural Phenotyping of Transgenic and Knockout Mice*. Wiley-Liss: New York, USA

Dawkins MS 1980 *Animal Suffering: The Science of Animal Welfare*. Chapman & Hall: London, UK

Duncan IJH 1993 Welfare has to do with what animals feel. *Journal of Agricultural and Environmental Ethics 6 (Suppl 2)*: 8-14

ETS number 123 1986 Strasbourg European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Available at http://conventions.coe.int/treaty/en/treaties/html/123.htm

European Union Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Available at http://europa.eu.int/comm/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf

European Union Council Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms. Available at http://dg3.eudra.org/F2/eudralex/vol-1/pdfs-en/900220en.pdf

Fraser D 1993 Assessing animal well-being: common sense, uncommon science. In: *Food Animal Well-Being* pp 37-57. Purdue University Office of Agricultural Research Programs: West Lafayette, USA

Fraser D 2003 Assessing animal welfare at the farm and group level: the interplay of science and values. *Animal Welfare 12*: 433-443

Fraser D, Weary DM, Pajor EA and Milligan BN 1997 A scientific conception of animal welfare that reflects ethical concerns. *Animal Welfare 6*: 187-205

Hagelin J, Hau J and Carlsson H-E 2003 The refining influence of ethics committees on animal experimentation in Sweden. *Laboratory Animals 37*: 10-18

Houdebine LM 2003 *Animal Transgenesis and Cloning*. John Wiley: Chichester, UK

Jegstrup I, Thon R, Hansen AK and Ritskes-Hoitinga M 2003 Characterization of transgenic mice — a comparison of protocols for welfare evaluation and phenotype characterization of mice with a suggestion on a future certificate of instruction. *Laboratory Animals 37*: 1-9

Kiley-Worthington M 1989 Ecological, ethological, and ethically sound environments for animals: toward symbiosis. *Journal of Agricultural Ethics 2*: 323-347

Mepham TB, Combes RD, Balls M, Barbieri O, Blokhuis HJ, Costa P, Crilly RE, de Cock Buning T, Delpire VC, O'Hare MJ, Houdebine LM, van Kreijl CF, van der Meer M, Reinhardt CA, Wolf E and van Zeller AM 1998 The Use of Transgenic Animals in the European Union: The Report and Recommendations of ECVAM Workshop 28. *Alternatives To Laboratory Animals 26*: 21-43

Pinkert CA 2002 *Transgenic Animal Technology: A Laboratory Handbook*, *2nd Edition*. Academic Press: London, UK

Rollin BE 1993 Animal welfare, science and value. *Journal of Agricultural and Environmental Ethics 6 (Suppl 2)*: 44-50

Royal Society 2001 *The use of genetically modified animals*. Policy document 5/01, UK

Stokstad E 1999 Humane science finds sharper and kinder tools. *Science 286*: 1068-1071

Thon R, Lassesn J, Hansen AK, Jegstrup IM and Ritskes-Hoitinga M 2002 Welfare evaluation of genetically modified mice — an inventory study of reports to the Danish Animal Experiments Inspectorate. *Scandinavian Journal of Laboratory Animal Science 1*: 45-53

van der Meer M, Rolls A, Baumans V, Olivier B and van Zutphen LF 2001 Use of score sheets for welfare assessment of transgenic mice. *Laboratory Animals 35*: 379-389

van Zutphen B and van der Meer M (eds) 1997 *Welfare of Transgenic Animals.* Springer Verlag: Berlin, Germany

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