GUIDELINES FOR ETHICAL CONDUCT WITH ANIMALS - COMPARATIVE GENOMICS OF MAN AND PIG

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Introduction

The objective of these ethical guidelines is twofold: to guarantee ethical conduct within the project and to prepare researchers to take up a dialogue with media and the general public concerning ethical questions that the project may give rise to. The guidelines also have two parts: this document in which we present the relevant ethical and animal welfare aspects of gene technology applications and give practical guidelines, and a small selection of additional texts which provide a more extensive theoretical overview of ethics of animal use and biotechnology applications on animals.

It is of course important that the work involving animals within the research project proceeds in a way that is ethically acceptable. Undoubtedly, ethically acceptable research procedures must follow current legislation for animal-based research: respecting the minimum requirements for animal housing, use of anaesthesia, treatment procedures etc. But we argue that for a researcher concerned with ethical aspects of his activity, it is equally important to strive to follow the general intentions of the law, such as attempting to find non-animal alternatives, trying to minimize the number of animals used etc.

Complying with legislation is a prerequisite for ethical conduct in research, but it becomes increasingly evident that it is not sufficient. Also research which is fully legal and licensed can be heavily questioned. When animal protection organisations question the way laboratory animals are treated, they are not satisfied with a reply that present regulations are followed. Likewise, many people are suspicious of research involving gene technology, even though the research comes from well-reputed laboratories which can be expected to comply with regulations in force. The reason for the critic or suspicion is a belief that the research may be ethically questionable, on grounds such as the harm done to the animals or the risks involved. The Comparative-genomics project involves both animal experimentation and gene technology, two issues which are highly controversial, and it is not unlikely that there will be criticisms against some of the planned activities. On the other hand, being controversial need not mean that activity in the project is unethical, since there are also arguments in favour of such research. It is a good idea for researchers to be prepared to present these arguments in an open dialogue with media and with the general public, if challenged. Being able to defend one's research in public of course requires making sure that the research is ethically reasonable, so researchers need to engage in some ethical evaluation of their own research activities (and be prepared to make changes if necessary). It is our hope that these guidelines should be of help in carrying out this ethical evaluation.

Presently, the use of animals for scientific purposes in Denmark is regulated through *Lov om dyreforsøg* (*Lov nr. 382 af 10. juni 1987*), implementing the *European Council Directive 86/609/EEC*.

In preparing these guidelines, we assume that the researchers are aware of and follow the law and will therefore not take up details of regulations which are to be found in the legislative texts.

1. **Ethics and welfare guidelines**

1. *It is essential that the intended experiments involving animals be subject to an ethics discussion beforehand. It is necessary to demonstrate the potential benefit of the experiments and that reasonable measures have been taken to protect the welfare of the experimental animals.*

Many people are sceptic towards the application of gene technology on animals. Important considerations are whether humans are entitled to interfere with the animal genome, environmental and health risks and finally implications for animal welfare. Because animal biotechnology applications are more controversial and give rise to more ethical concerns than most scientific activities, it is particularly important that scientists in this field consider the ethical aspects of their activity. That there is an expected benefit of the experiment is of particular importance when the ethical concerns are many, as in biotechnology projects. If an acceptable alternative method is available, the benefit of creating a genetically modified animal is limited. If a mouse model already exists, or is easier to develop than a pig model, there will be little additional benefit of developing a pig model. We suggest that scientists in the present project, which intend to do experiments involving living animals, enter in contact with us in the ethics group to discuss these plans.

2. *Biotechnology applications may result in more or less severe animal welfare problems. Before embarking on a project involving animals, the scientists should attempt to predict the expected problems, based on existing information about the species used and the gene(s) involved. This prediction should be presented at the ethics discussion.*

Modification of the genome will result in animals with an altered phenotype. At present, the most common applications in the laboratory involve inactivating genes or replacing them with mutated versions and the usual result is an animal in which one or several functions have been disrupted. One can expect such disruptions to have more or less profound effects on animal welfare. In fundamental biology research, when the function of the gene in question is partly unknown, the outcome of producing animals with under-expression or over-expression of that specific gene is rather unpredictable. The produced animals may be severely impaired, with considerable welfare problems, or they may be seemingly unaffected by the manipulations. More information is available in the case of developing animal models of human disorders, where knowledge about the symptoms in humans gives some indication of which welfare problems to expect (although considerable uncertainty remains). Welfare can also be negatively affected through side effects of the developed characteristics (as when increasing production in farm animals lead to an increase in production diseases). Danish researchers have reported welfare problems in about 30 % of the produced transgenic or knock-out mouse strains have considerable welfare problems (see Jegstrup et al 2003). Given how difficult it is to predict which strains will develop problems, it is sensible to adopt a more cautious attitude and treat the development of each new strain as a potential welfare problem (for example, the Canadian Animal Care and Use Committees consider development of a new strain as an experiment of severity category D, that is one which causes moderate to severe distress or

discomfort). Whether or not welfare problems exist can then be confirmed through careful examination of the animals, as will be discussed in the following section.

3. ***When a new line of genetically modified animals is being developed, the welfare of the animals should be closely monitored following a pre-defined protocol.***

Even though estimates can be made *a priori,* evidence of the function of a gene under study comes only when the genetically modified animals are born and their phenotype can be studied. Many of the potential welfare problems will be present when the animals are born, so it is important that they are detected as soon as possible. When producing new strains of genetically modified mice, the animals are closely monitored during the first weeks after birth, precisely to determine the effect of the genetic modification (phenotyping). Combining the phenotype characterization with schemes of appropriate welfare parameters is a useful way to detect welfare problems at an early stage. A number of different schemes for this purpose have been described for mice (see Jegstrup et al 2003 for an overview). Observations according to these schemes include a number of different characteristics, such as physiology, morphology, neurological capacity and behaviour. The schemes also establish a schedule for when different observations or tests should be made, and in some cases suggest actions to take if welfare problems are detected. The search for welfare problems should not be restricted to the early postnatal period, because some problems appear only later in the life of the animals (such as in animals prone to developing tumours) or may not appear until later generations. If the animals are to be kept and studied for a longer period of time, it is important that their welfare is continuously monitored. Welfare assessment protocols developed for mice will have to be adapted for pigs, in case genetically modified animals are being produced, and the animal ethics group can assist with this. We suggest the protocol developed by Mertens and. Rülicke (2000) as a starting point.

4. ***Humane endpoints should be defined for the state where the welfare of an animal is unacceptably low and actions must be taken. A protocol for humane endpoints should describe how the animals should be monitored, at which symptoms the endpoint should be applied and which action should be taken (usually euthanasia or treatment of the animal).***

Endpoints are predefined points beyond which the experiment is not allowed to proceed. Such endpoints which are determined with the objective of reducing animal suffering are sometimes called 'humane endpoints' (eg Morton 1999). These endpoints are defined in terms of the symptoms that the animals show, and usually the decision is to euthanize the animals once the endpoint is reached. Alternatively, if an appropriate therapy is available, it can be decided to start applying this therapy at the endpoint. The experiment should be interrupted or the animal taken out of the experiment or treated when it is recognised that an individual experiences pain, suffering, distress or lasting harm beyond what is acceptable. Four criteria are essential in order for the refinement through endpoints to be successful: that the definition of the endpoint is chosen with careful attention to relevant welfare measures, that the animals are closely and frequently monitored, that the method used allows to detect relevant signs and that the personnel monitoring the animals have received the appropriate training. A number of schemes for detecting distress and suffering in research animals have been suggested, and useful information can be found on-line, for example in

the ILAR Journal vol 41(2) http://www4.nas.edu/cls/ijhome.nsf/. Because the endpoints should be adapted to the specific problems in the experiment in question, it may be useful to first perform a pilot study on a small number of animals before defining the endpoints and protocol to be applied to the larger group of animals produced.

5. *After the welfare consequences of the genetic modification have been identified, housing and husbandry routines should be adapted to cater for the special needs of the animals*

Some of the genetic modifications result in phenotype alterations which give rise to welfare problems, which can to some extent be overcome through adapting the way these animals are housed and handled. The case of immuno-compromised animals is well-known – these animals are housed in isolators or individually ventilated cages to avoid undesired infections. Problems in animals which increased aggressive tendencies can be prevented if changes of group composition are avoided or if necessary by housing animals individually. Animals which are more vulnerable to stress can be housed in a room where there is less movement, and in strains with disturbed maternal behaviour it may be necessary to foster the offspring to a wild-type strain. There are many examples of animals which have special needs and housing and husbandry should be adapted considering each line as a specific case.

6. **It is important that scientists doing research on animal biotechnology have a basic understanding of the different ethical and animal welfare aspects of their activity, to aid their own ethical decision-making and to help them participating in the debate in society**

Animal experimentation is one of the most controversial issues in the discussion about animal use, and animal biotechnology has given rise to even more discussion in society than 'traditional' animal experimentation (e g Olsson & Sandøe, in press). The image most people have of animal experimentation is based on the disturbing photos presented by animal rights organisations. People in general are unaware of the fact that most laboratory animals are not subject to such invasive treatments, and that many are not exposed to painful stimuli at all. Neither are they aware of the role of animal experimentation in advancing knowledge and developing treatments for human and animal ailments. The only way to get an informed public discussion over animal use is to get all stakeholders involved in it. This means that scientists must take part in the debate, just as the animal protection organisations already are doing. However it is not only important to participate but also to be able to argue in an appropriate way, to be able to articulate one's own view and base it on a solid ethical argumentation.as well as to understand the motives and arguments of the opponent. Insight in ethical theories and understanding of ethical analysis can help to guide an informed and rational discussion of how animals should be treated, perhaps even resulting in a compromise upon which all can agree. In the second part of this document, we suggest some basic reading for an introduction to ethics of animal experimentation in general and animal biotechnology in particular. We also suggest that scientists take part in interdisciplinary seminars, where different ethical aspects of research can be discussed.

Checklist for projects involving living animals

For the present and future projects of the same kind, we propose that the research groups which will be producing genetically modified animals have a thorough discussion of animal welfare and ethics aspects of their proposed research, together with the members of the animal ethics group and the responsible laboratory animal veterinarian. Useful questions to address could be:

Benefits of the experiment
How important are the expected benefits from this experiment?
How likely is it that these benefits will be obtained?
Which methodologies could be used to obtain the benefits?
(Why) Is the proposed methodology perceived as the best?

Consequences for the animals
Which is the expected harm done to the experimental subjects?
How long will the animals be exposed to this harm?
How many animals will be used?
Which methods are available to reduce animal distress? How can these methods be applied?
How will the animals be monitored?
Which criteria for endpoints will be applied?

We also propose that this discussion should result in the following documents, of which the first should be available as soon as genetically modified animals are being produced and the second two after an initial observation of the first animals has indicated the main problems these animals experience:
- protocol for early detection of welfare problems
- protocol for housing and husbandry procedures to cater for the special needs of impaired animals
- criteria for endpoints and a protocol for monitoring animals

Ethics and Animal Welfare

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Animal welfare - Practical aspects

Overview of welfare assessment protocols
- Jegstrup I, Thon R, Hansen AK & 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. [1]

The Swiss welfare assessment protocol
- http://www.forschung3r.ch/en/publications/bu19.html
- Mertens C & Rülicke T. 2000. Phenotype Characterization and Welfare Assessment of Transgenic Rodents (Mice). Journal of Applied Animal Welfare Science 3(2); 127 - 139.

Humane endpoints
- ILAR Journal vol 41(2) http://www4.nas.edu/cls/ijhome.nsf/.
- Morton DB. 1999. Humane endpoints in animal experimentation for biomedical research: ethical, legal and practical aspects. In: Hendriksen CFM, Morton DB (eds) Humane endpoints in animal experiments for biomedical research. London:Royal Society of Medicine Press. *(These symposium proceedings are available on-line at* http://www.lal.org.uk/onlinerep.html *)*