CCAC guidelines on: transgenic animals (1997)

The Canadian Council on Animal Care (CCAC) is responsible for the oversight of animals used in research, teaching and testing. In addition to the *Guide to the Care and Use of Experimental Animals*, Vols. 1 and 2, which lay down general principles for the care and use of the animals, the CCAC also publishes guidelines on issues of current and emerging concerns. The CCAC *guidelines on: transgenic animals* is the second of this series and has been produced by the scientific subcommittee of the CCAC. The creation and use of genetically modified animals is a rapidly evolving field of research, therefore, these guidelines will be subject to regular review.

The following guidelines for transgenic animals are provided: to assist Animal Care Committee (ACC) members and investigators in evaluating the ethical and technological aspects of the proposed creation, care and use of transgenic animals; to ensure that transgenic animals are used in accordance with the CCAC statement *Ethics of Animal Investigation*; and to ensure that the well-being of Canadians and the environment are protected.

By definition, the term "transgenic animal" refers to an animal in which there has been a deliberate modification of the genome - the material responsible for inherited characteristics - in contrast to spontaneous mutation (FELASA, September 1992, revised February 1995). Since 1981, when the term "transgenic" was first introduced by J.W. Gordon and F.H. Ruddle, genetically-engineered animals have become increasingly important as research subjects.

Transgenic animals are used: in the basic biological study of regulatory gene elements; in medical research, to identify the functions of specific factors in complex homeostatic systems through over- or under-expression, as models of human disease; in toxicology as responsive test animals; in biotechnology as producers of specific proteins; and in agriculture and aquaculture to improve yields of meat and other animal products. This list is not inclusive; the use of transgenic animals is likely to expand in the future.

There are three main methods used for the production of transgenic animals: DNA microinjection; retrovirus-mediated gene transfer; and embryonic stem (ES) cell-mediated gene transfer. DNA microinjection is the first method that was developed and provides the underlying concept for the other two methods. The introduced DNA may lead to the over- or under-expression of certain genes or to the expression of novel genes. The integration of the introduced gene into the host DNA, which is accomplished by the microinjection of DNA into the pronucleus of a fertilized ovum, is a random process and the introduced gene will not necessarily insert itself into a site that will permit its expression. Therefore, other methods have been devised, including vector-mediated gene transfer and homologous recombination, to increase the probability of expression. Retro-viruses are commonly used as vectors to transfer genetic material into the cell. The third method uses homologous recombination of DNA to permit precise targeting of DNA sites in embryonic stem cells. If the homologous sequence to be introduced into the cell, carries a mutation or a gene from another species, the new sequence will replace the specific targeted gene. This procedure is the method of choice for gene inactivation, the so-called "knock-out" method and is of particular importance for the study of the genetic control of developmental processes.

Transgenic animals provide the investigator with an extremely powerful tool for the development of disease models, since the mechanisms of gene regulation will receive a greater understanding. In addition, the use of transgenic mouse models which more closely

mimic the human disease can replace the need to use more sentient animals as models. The better specificity of models may in time also lead to a reduction in the number of animals used. Genetic modification of livestock may also be seen as a benefit to human health, in the economic and efficient production of important pharmaceutical proteins.

In parallel with the development of transgenic technology, ethical concerns have arisen about the use of this technology. These concerns are wide ranging and encompass animal welfare, human health and environmental issues. They include animal suffering caused by the expression of transgenes inducing tumors or neurodegenerative diseases, etc., the possible escape of transgenic animals into the environment, not to mention the possibility for the modification of the human genome.

The production and use of transgenic animals are subject to all of the considerations raised by the CCAC *guidelines on: animal use protocol review*. Protocols must, therefore, be reviewed in the same manner. However, a close look must be given to the procedures involved and in particular to possible welfare concerns for the progeny from transgenic animal creation protocols. For these reasons the guidelines also require a *Transgenic Information Sheet* (Appendix) to be completed with the protocol submission.

In implementing these CCAC guidelines, ACCs and investigators considering the welfare of the animals in the proposed study will have to take into account the special features of each transgenic strain. In addition, they will have to be sensitive to ethical concerns and alert to technological changes in this rapidly evolving field. The CCAC anticipates that modifications to the guidelines will be required as this evolution occurs.

- **Investigator and Animal Care Committee Responsibilities**

a. **Education**

It is the responsibility of the ACC to ensure that all its members are informed about the ethical and technological aspects of transgenic animal use. A suggested reading list is attached. It is also recommended that researchers applying for ACC approval to create or use transgenic animals be conversant with ethical concerns surrounding the use of these animals, and be prepared to justify their work as being in the public interest.

b. **Proposals to create new transgenic strains**

i. Standard procedures for creating transgenic animals can be dealt with by ACCs according to their usual practices for surgical procedures.

ii. In reviewing applications for creation of novel transgenic animals, ACCs should determine that:

- the investigator has competent technical assistance and experience in the necessary record-keeping for breeding colony maintenance;
- arrangements for surgical procedures, colony housing and maintenance, have been discussed with and approved by the local Animal Facility Management;
- the investigator and the technical staff involved in daily monitoring of the transgenic colony are familiar with signs of distress in the species of study;

- a frequent, reliable, thorough, and documented monitoring system is in place to detect behavioral, anatomical and physiological abnormalities indicative of animal distress; and
- endpoints for survival are clearly defined.

Standard operating procedures (SOPs) can be developed to deal with these concerns.

iii. Proposals to create or use transgenic animals should include information about expected phenotype (as indicated in the Appendix), to include information about anticipated pain or distress levels in the transgenic animal, measures which will be taken to alleviate such distress, and the required monitoring system.

iv. Proposals to create novel transgenics initially should be assigned CCAC category of invasiveness level "D" . If approval is merited, it should be provisional, limited to a 12-month period, and subject to the requirement that the investigator report back to the ACC as soon as feasible on the animals' phenotype, noting particularly any evidence of pain or distress.

After receiving the report from the investigator, the ACC may confirm approval of the proposal and adjust the level of invasiveness. However, if the animals are noted to be suffering unanticipated pain or distress, the ACC will ask the investigator to provide a revised protocol which will minimize and alleviate distress, and will reconsider its approval of the proposal.

c. **Proposals to utilize existing transgenic strains**

i. A proposal on transgenic animals may have two parts: creation of the transgenic animals, and subsequent experimental manipulations of the animals. Except where subsequent manipulations are restricted to observation and euthanasia of the transgenic animal, creation and use proposals should be considered as separate proposals.

ii. In reviewing use proposals, ACCs should consider whether procedures regarded as acceptable in non-transgenic animals, are still acceptable in transgenic animals where altered phenotype may impose additional stresses.

iii. Proposals to use existing transgenic strains should also include the information requested in the Appendix.

d. **Accounting**

i. Estimates of all animals to be used or generated in a transgenic study should be stated in proposals to the ACC, listed by use category (e.g., oocyte donors, pseudopregnant females, male "studs", successful transgenics, etc.)

ii. When completing the *Animal Use Data Form* for reporting annual animal usage to CCAC, investigators should identify transgenic animals separately from non-transgenic animals in the "Species" column.

iii. To reduce overall animal use, CCAC encourages, when appropriate, assignment of non-transgenic animals, bred in a transgenic creation procedure, to other ACC-approved protocols. Asymptomatic heterozygotes must be clearly identified and should only be used for breeding purposes when the investigator is aware of their altered genotype. Accounting procedures within animal facilities must prevent double-counting of such transferred animals in annual use statistics.

e. **Containment**

i. All proposals for creation or use of transgenic animals must assure the ACC that risks to human health and the environment are minimized to an acceptable level. For transgenic animals created using micro-injection or replication-defective viruses, the containment risks are limited to those associated with the escape of the animal and interbreeding with wild stocks. Proposals should include information about:

- o containment and security procedures in animal facilities and, if applicable, during transportation when importing the animal;
- o plans for recapture should a breach of containment occur; and
- o the consequences to human health or wild populations should containment fail.

ii. For commonly-used transgenic species, each animal facility should have SOPs for containment, which can be referenced by proposals.

iii. ACCs should discuss with the institutional Biohazard Committee any proposal which raises biohazard containment concerns.

f. **Other regulations**

i. ACC approval of a proposal does not relieve the investigator of responsibility to satisfy the regulations of any other governmental agencies. For example, creation of any transgenic fish strain requires approval of the Department of Fisheries and Oceans. Biohazard approval may also be required for some proposals.

- **Responsibilities of CCAC**

a. **Education**

To update at least every two years a reading list on ethical and technical aspects of transgenic animal use which can be distributed to members of ACCs, this list to include articles appropriate for all members.

b. **Accounting and reporting**

To include in its annual animal usage statistics separate totals for transgenic strains of each species used in experiments.

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Reading List

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APPENDIX

Transgenic Information Sheet

1. Species: _____
   Background Strain: _____
   Heterozygotes: _____
   and/or
   Homozygotes: _____

2. To be housed in Room _____.

3. What is the health profile of the source colony? Provide the most recent serology report.

4. What known traits will affect breeding and lifespan?

5. What abnormalities are known to exist (or do you expect) in these animals?

6. If you expect these abnormalities will cause pain or distress, how will you minimize or alleviate it?

7. Describe your monitoring and recording procedures for detecting physical or behavioral abnormalities which are indicative of pain and distress.

8. What objective criteria will be used to determine if an animal will be removed from the study prematurely?

9. If biological containment is required, state reasons and the level required. Describe your containment and security procedures. How will you deal with breach of containment? Will there be any risks to human health, wild populations or the environment generally if containment fails?

10. If you are generating a novel transgenic strain, provide a timetable for this process and indicate when you expect to report back to the ACC on the phenotype obtained.

***Note**: Items on this list may be covered in the regular protocol form of the ACC, and do not need to be duplicated. Where institutional standard operating procedures have been developed (e.g., for containment), refer to these.*