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# Guidelines development and scientific uncertainty: use of previous case studies to promote efficient production of guidelines on the care and use of fish in research, teaching and testing

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## Abstract

*The Canadian Council on Animal Care (CCAC) develops guidelines on issues of current and emerging concern in response to the needs of the scientific community, advances in animal care, and the needs of the CCAC Assessment Program. Guidelines are developed by subcommittees of experts, and are based on sound scientific evidence. However, the process of guidelines' development can involve consideration of areas where there is little scientific certainty or where scientific evidence needs to be tempered by other ethical considerations. Often these are areas where recommendations to the community are most needed, to provide assistance to both investigators and animal care committees on how best to balance the well-being of experimental animals and the goals of scientific research. The process for drafting the CCAC* guidelines on: the care and use of fish in research, teaching and testing *(in preparation) will be used as an example of the development of guidelines in the face of uncertain science, alongside a discussion of the CCAC* guidelines on: transgenic animals *(1997), as an example of the employment of a precautionary approach. Fish are now one of the most commonly used laboratory animals in Canada. However, what constitutes well-being for fish is an emerging field with often conflicting scientific data, and this presents unique challenges in guidelines' development.*

**Keywords**: *animal welfare, ethics, fish, genetically modified animals, pain and distress, precautionary approach*

## Introduction

The Canadian Council on Animal Care (CCAC) is a national organisation with the responsibility for overseeing the care and use of animals in Canadian science. The CCAC system is built on three interrelated programs: the Assessment Program, the Education and Training Program, and the Guidelines Development Program. CCAC guidelines are developed on issues of current and emerging concern in response to the needs of the scientific community, advances in animal care, and the needs of the CCAC Assessment Program. Two principal audiences are targeted by the guidelines: investigators, who require information on the care and maintenance of animal subjects as well as on the ethical acceptability of procedures to be carried out; and animal care committees (ACCs), responsible at the local level for monitoring animal care and use.

## Guidelines development process

The CCAC is a peer-based organisation involving scientists, veterinarians and community representatives at all levels of its operation. Guidelines are developed by subcommittees of experts, peer-reviewed by additional pools of experts, both nationally and internationally, and subject to a widespread review involving constituents of the CCAC Program and any parties likely to be affected by the guidelines. In addition, the CCAC Guidelines Development Program is responsible for international harmonisation of guidelines while ensuring that the guidelines meet the requirements of the Canadian context.

## The CCAC and the Three Rs

The principles of the Three Rs (Replacement, Reduction and Refinement), first outlined by Russell and Burch in 1959, have become enshrined in legislation regulating the use of animals for scientific purposes in several countries. In Canada, where there can be no federal legislation in this area because of the Constitutional division of power (Wilson 1998), the CCAC, as the national quasi-regulatory body, has incorporated these principles into its fundamental policy document *Ethics of Animal Investigation* (CCAC 1989). For the CCAC, the principles of the Three Rs are stated as:

> "The use of animals in research, teaching, and testing is acceptable ONLY if it promises to contribute to understanding of fundamental biological principles, or to the development of knowledge that can reasonably be expected to benefit humans or animals. Animals should be used only if the researcher's best efforts to find an alternative have failed. A continuing sharing of knowledge, review of the literature, and adherence to the Russell–Burch '3R' tenet of 'Replacement, Reduction and Refinement' are also requisites. Those using animals should employ the most humane methods on the

smallest number of appropriate animals required to obtain valid information."

The CCAC *Ethics of Animal Investigation* (CCAC 1989) requires that any use of an animal be of benefit to society. Investigators are not required to weigh the harms to the animals against the potential benefits to society *per se*; however, this requirement is implicit in guidelines developed to provide assistance to both investigators and ACCs on how best to balance the well-being of experimental subjects and the goals of scientific research. The limits on harms are further set in the *Ethics of Animal Investigation* where certain procedures are deemed to be unacceptable (for example, the use of muscle relaxants or physical trauma without anaesthesia) and where special caution is required for particular types of studies (for example, studies on stress and pain, or those involving food and water restriction). Further limits on harms have been established, as has a process for establishing endpoints to minimise pain and distress, through the CCAC *guidelines on: choosing an appropriate endpoint for experiments using animals in research, teaching and testing* (CCAC 1998).

CCAC guidelines are first and foremost based on sound scientific evidence. In line with policy generated by the Guidelines Committee (one of the five standing committees of the CCAC, which is responsible for overseeing the Guidelines Development Program), every guideline statement should be fully justified, including, as far as possible, reference to the published literature. In addition, the iterative process of CCAC guidelines' development ensures that recommendations made by expert members of the subcommittee responsible for the development of the guidelines' document are subject to peer review by an additional group comprising both national and international experts in the area, plus a further review by individuals and organisations likely to be affected by the guidelines.

In accordance with the principles of the Three Rs, CCAC guidelines seek to provide recommendations that minimise pain and distress arising as a result of experimental procedures carried out on animals, as well as recommendations that focus on improving animal well-being through meeting the psychological, social and behavioural needs of animals. However, often the scientific basis needed to provide an understanding of the impact of procedures (or housing and husbandry) on animal well-being is in itself the focus of an emerging area of research. The contexts in which animals are used, whether in the area of biomedical, agricultural, or ecological research, shift more rapidly than the associated welfare-orientated research. For example, there has been a rapid increase in the use of genetically modified animals prior to a complete understanding of the potential for phenotype abnormalities, and an increase in the use of fish as a research model prior to a complete understanding of the housing preferences of different species.

Strategies which have been employed previously during guidelines' development, and which seek to address the lack of scientific evidence in order to minimise (and where possible eliminate) pain and/or distress, or in order to meet the needs of animals in a laboratory setting, can be useful to subcommittees embarking on the development of new guidelines. One recommended approach, elaborated at the time of the development of the CCAC *guidelines on: choosing an appropriate endpoint in experiments using animals in research, teaching and testing* (CCAC 1998), was the requirement for a pilot study involving only a small number of animals in cases where the outcome of a particular procedure was unknown. The following sections of this paper describe a strategy developed by the CCAC's scientific subcommittee during the development of the CCAC *guidelines on: transgenic animals* (CCAC 1997) to provide protection for animals when the outcome of manipulating an animal's genome is unknown. We also discuss how this strategy is currently being employed by the CCAC subcommittee on fish, in the development of CCAC *guidelines on: the care and use of fish in research, teaching and testing* (in preparation).

## The notion of harm for genetically modified animals

In a report to the Government of Canada entitled *Patenting of Higher Life Forms and Related Issues*, the Canadian Biotechnology Advisory Committee (CBAC) addressed the issue of 'ordre public' or morality provisions in international patent law (CBAC 2002). The CBAC reached the conclusion that "with regard to research and experimentation involving animals, by the time a researcher is in a position to file for a patent, any inappropriate harm to the animal resulting from the research will already have been done" (CBAC 2002, p 37). Hence the Canadian *Patent Act* (1985) can have little if any effect in such situations. It is therefore of great importance to have sufficient controls in place, both upstream and downstream of the reach of the *Patent Act*, in order to provide regulation for the creation and use of genetically modified (GM) animals (Gauthier & Griffin 2000). In this respect, the CCAC's ethical review system is designed to integrate the needs of scientists, animals and the community at the local level in a proactive way, and to set standards for the care and use of animals in science at the national level.

The *Report of the Committee to Consider the Ethical Implications of Emerging Technologies in the Breeding of Farm Animals* (Banner 1995) challenged the tendency to assess new technologies solely in terms of questions regarding risk and benefits. In doing so, it outlined three basic principles:

(1) "Harms of a certain degree and kind ought under no circumstances to be inflicted on an animal."

(2) "Any harm to an animal, even if not absolutely impermissible, nonetheless requires justification and must be outweighed by the good which is realistically sought in so treating it."

(3) "Any harm which is not absolutely prohibited by the first principle, and is in particular circumstances considered justified in the light of the second, ought to be minimised as far as is reasonably possible."



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## The CCAC *guidelines on: transgenic animals* (1997)

Throughout the 1990s there was a rapid increase in the creation and use of GM animals. To illustrate this, a search was made of *Medline*, an online database of biomedical journals, to ascertain the number of papers published per year, for each year from 1990 to 1999 using the keywords: 'genetic susceptibility to disease'; 'hybridoma', 'xenotransplantation'; and 'genetically modified' ('transgenic' and 'knock-out') animals (see Figure 1).

In 1997, faced with an increasing number of protocols involving the creation and use of GM animals, the CCAC published *guidelines on: transgenic animals* (CCAC 1997). These guidelines were a first step in providing protection for the GM strains that were being created within Canadian universities.

At the time of the development of these CCAC guidelines, the subcommittee had limited scientific evidence on the effects of insertion of genes into an animal's genome, or gene inactivation. It recognised that the difficulty for the application of genetic understanding lay in the lack of complete knowledge of the consequences of investigators' interventions and manipulations (Banner 1998). While the subcommittee agreed with the first principle of the Banner Report (Banner 1995), in practice it is difficult to determine prior to a genetic manipulation whether or not the manipulation will lead to unacceptable levels of harm.

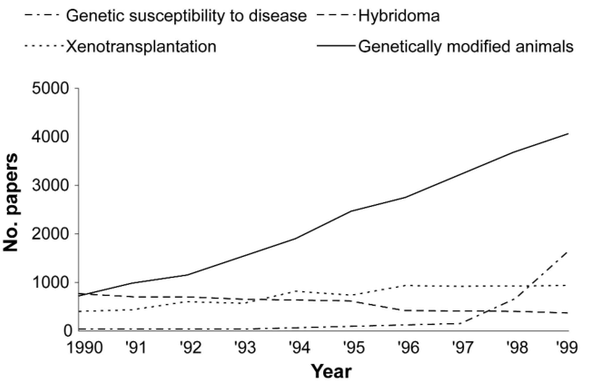
Genetically modified animals were viewed as providing the investigator with a powerful tool for developing disease models because they would increase understanding of the mechanisms of gene regulation. In addition, it was thought that the use of GM mouse models that more closely mimicked human disease would, in time, reduce the need to use more sentient animals as models, and that the increased specificity of models would lead to a reduction in the number of animals used.

However, it was also recognised that, in parallel with developments in biotechnology, there are ethical concerns about the use of GM technology. These concerns are wide ranging and encompass not only animal welfare, but also human health and environmental issues. In part, these concerns are difficult to address because of the necessity for action in the light of limited scientific or empirical information. In terms of animal welfare, the CCAC subcommittee was aware that genetic modification could not be regarded as a single moral entity: some genetic modifications may be intrinsically objectionable as manipulative of an animal's good, some may not, some may be neutral in relation to an animal's welfare, while some may actually result in improved welfare, and others may do severe harm (Banner 1998).

The subcommittee recognised that in implementing the CCAC *guidelines on: transgenic animals* (CCAC 1997), ACCs and investigators would have to take into account the special features of each transgenic strain. The guidelines reflect this necessity in adopting a precautionary approach.

At the outset it was understood that, in line with the CCAC *Ethics of Animal Investigation* (CCAC 1989), any animal observed to be experiencing severe unrelievable pain or distress would be euthanased. The CCAC *Guide to the Care and Use of Experimental Animals* (CCAC 1993, Chapter 10) provides key indicators of pain and distress for the main species of animals used in research, teaching and testing.

**Figure 1**




Trends in the worldwide use of animals in four fields of biomedical research. Data are based on the number of publications appearing in journals included in *Medline*, an online database of biomedical journals, between 1990 and 1999 using keyword searches on: 'genetic susceptibility to disease'; 'hybridoma'; 'xenotransplantation'; and 'genetically modified animals' (from Griffin & Gauthier 2000).

Two particularly important elements were included in the guidelines:

(1) "Proposals to create or use transgenic animals should include information about expected phenotype, 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" (CACC 1997, p 3).

Investigators are required to complete an additional transgenic information sheet or to ensure that all of these elements are covered in their protocol submission to an ACC.

(2) "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 requirements that the investigator report back to the ACC as soon as feasible on the animals phenotype, noting particularly any evidence of pain or distress" (CCAC 1997, p 3).

## Categories of invasiveness

The CCAC requires that every protocol be assigned to one of five categories of invasiveness, which range from Category A (least invasive) to Category E (most invasive) (CCAC 1991). Category D protocols are defined as "Experiments which cause moderate to severe distress or discomfort — eg major surgical procedures conducted under general anaesthesia with subsequent recovery;



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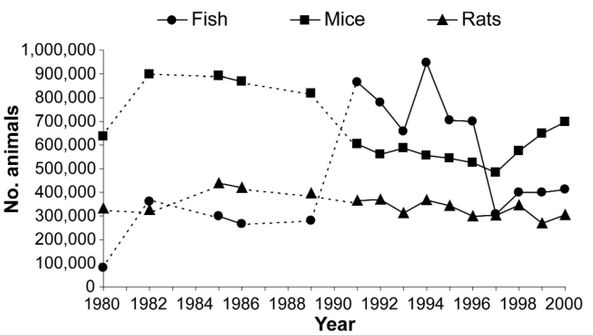
**Figure 2**




Trends in the number of fish, mice and rats used in research, teaching and testing in Canada between 1980 and 2000. In 2000, animals of these taxa represented 72.4% of all animals used. (Dotted lines indicate years for which no data are available.)

prolonged (several hours or more) periods of physical restraint; induction of behavioural stresses such as maternal deprivation, aggression, predator–prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organisation; the use of Freund's Complete Adjuvant" (CCAC 1991, p 2).

Requiring that protocols involving the creation of GM strains be placed in Category D advocates a precautionary approach. It is likely that many of the GM strains developed will show few, if any, signs of adverse welfare. However, the CCAC has always emphasised the 'potential' for pain and distress as the key factor in assigning categories of invasiveness. This has the additional advantage of encouraging ACCs to pay particular attention to those protocols where pain and distress could be a concern.

As an aside, it should be noted that this approach has an influence on the annual reporting of animal use data. Comparison with data from countries which record levels of pain and distress 'experienced' by the animals, may lead to the assumption that a higher proportion of animals actually experience pain and distress in Canada (Gauthier in press).

The requirement for investigators to assign protocols concerning the creation of GM animals to a high level of invasiveness, with subsequent reclassification being permitted following submission to the ACC of information on the phenotype and any associated problems, means that the animals must be closely monitored. In this respect, evaluation of the well-being of the genetically modified strains, once created, would be assisted by a formalised well-being assessment chart (Jegstrup *et al* 2003). This will be incorporated in the revised CCAC *guidelines on: transgenic animals*. A framework for the evaluation of the well-being of GM agricultural livestock is also currently under discussion by the Canadian Government.

## Patterns of animal use

All species of vertebrate are covered by the CCAC, as well as cephalopods. Figure 2 provides an illustration of the relative numbers of fish, mice and rats used in research, teaching and testing in Canada. These are the most commonly used animals.

Mice accounted for the vast majority of animals used in Canada until 1991, when fish became the most used taxon. Extensive molecular biology experiments, among others, required the use of an increased number of mice through the 1980s with a subsequent return to former levels in the 1990s when the use of *in vitro* methods increased. Between 1991 and 1997 the use of mice decreased continuously, before starting to increase due to increasing use of GM animals. It is interesting to note that the number of fish used remained elevated between 1991 and 1996, before decreasing abruptly in 1997. This transient increase in the use of fish corresponds with the enforcement of the *Canadian Environmental Protection Act* of 1988 (revised in 1999) and the resulting transient need to perform more regulatory testing.

## CCAC *guidelines on: the care and use of fish in research, teaching and testing*

The CCAC *guidelines on: the care and use of fish in research, teaching and testing* are currently being developed. These guidelines have already undergone one level of peer-review by experts, and a further widespread review in the summer of 2003.

A large proportion of the guidelines will focus on practical aspects for fish well-being such as facilities, water quality and standards for surgical procedures, and are not discussed here. Readers are encouraged to consult the CCAC website to access the second draft and final publication once posted (http://www.ccac.ca).

In striving to produce a document that will encourage the ethical consideration of fish as a research animal, the CCAC subcommittee developing the guidelines has given considerable thought to the potential for fish to experience pain and distress. However, the subcommittee struggled with the same difficulties outlined by the Fisheries Society of the British Isles (FSBI) in their briefing paper: "The scientific study of welfare is at an early stage compared to work on other vertebrates and a great deal of what we need to know is yet to be discovered" (FSBI 2002, p 3).

It is generally accepted that mammals experience distress, discomfort and pain, and efforts are increasingly being focused on the recognition of pain and distress in laboratory animals (Hawkins 2002). There are authors, nonetheless, that continue to challenge claims that non-human species have the capacity to experience pain. Bermond (1997), for instance, has argued that because conscious awareness depends on extensive development of the frontal lobes, few, if any, mammals besides humans possess adequate cortical substrate for pain experience. It is important to know whether or not fish can experience pain, because this may have an influence on perceptions of how these animals should be managed.

Rose (2002), in a review of the literature, came to the conclusion that fish do not have the capacity to experience pain. He based his conclusion on three points:

(1) Behavioural responses to noxious stimuli are separate from the psychological experience of pain.

(2) Awareness of pain in humans depends on functions of specific regions of the cerebral cortex.



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(3) Fish lack these essential brain regions or any functional equivalent, making it untenable that they can experience pain.

Pain in humans has been defined as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage" (IASP 1979). However, the assessment of an animal's emotional experience is impossible. Therefore Bateson (1992), amongst others, has argued that emotion should not feature in the definition of pain in animals. It is most likely that what an animal 'feels' as pain is nothing like that experienced by humans with their more complex brain structure; however, that does not mean that the animal's experience is not unpleasant. Key to the discussions of the CCAC subcommittee was the level of importance that should be given to pain and distress for fish, both in terms of their biology and the ethical consideration they should be given. Determining when fish are in pain or distress is problematic, but an incomplete understanding of pain, nociception and distress in fish does not mean that the issue can be ignored.

The CCAC subcommittee considered factors such as the neuroanatomy of fish. They noted that, although fish do not have a neocortex (the area of the brain considered to be responsible for conscious awareness of pain), the telencephalon of the fish brain has been shown to have sensory and higher order functions such as avoidance learning (Overmeir & Papini 1986). The subcommittee was therefore reluctant to conclude that there is no central processing of nociception. In the rainbow trout (*Oncorhynchus mykiss*), anatomical examination of the trigeminal nerve has identified two types of nociceptor: A delta and C fibres known to be associated with pain perception in mammals (Sneddon *et al* 2003). In addition, jawed fish are known to produce some of the natural opiates that are involved in nociception in mammals (Vecino *et al* 1992; Rodriguezmoldes *et al* 1993; Zaccone *et al* 1994; Balm & Pottinger 1995).

Based on this scientific evidence, the CCAC subcommittee decided to adopt the approach that fish exhibit the potential to perceive pain, and therefore issued the following guideline: "Fish have the potential to experience pain and manipulations that provoke stress or avoidance/escape behaviour may be causes of distress. Researchers have a moral obligation to mitigate or minimise potential pain and distress whenever feasible and consistent with good scientific practice."

These guidelines also assert that the use of any animal, including fish, for the purpose of research, teaching or testing, will be accorded more emphasis on well-being than is generally accepted for the killing of animals for food.

Recently, there have been some elegant studies carried out on pain perception in fish by Sneddon, Braithwaite and colleagues (Sneddon *et al* 2003; Braithwaite & Huntingford 2004, pp 87–92, this issue). These studies try to tease apart the elements required to demonstrate that an animal is capable of pain perception. In order to do so, it is necessary to demonstrate that the animal perceives an adverse sensory stimulus and then reacts to it both physiologically and behaviourally. In addition, to verify that this is not a simple nociceptive reflex, it is necessary to show that the animal learns that the stimulus is associated with an unpleasant experience and subsequently avoids it.

Sneddon and colleagues (2003) were able to show electrophysiologically that there are nociceptors present in the lip region of rainbow trout. Injection of bee venom or acetic acid into the lips of trout resulted in the avoidance of feeding and anomalous behaviours (eg rocking behaviour and rubbing of affected area) that were considered to be indicative of pain perception, as well as physiological responses (eg increase in opercular rate, exceeding the increase in rate generally associated with handling stress). The types of studies conducted by Sneddon and her colleagues will assist in the further development of the CCAC guidelines because they provide further data needed to ensure that the guidelines are grounded in scientific evidence.

## Conclusion

CCAC guidelines are developed in response to current and emerging concerns to meet the needs of the scientific community and the CCAC Assessment Program. While the CCAC Guidelines Development Program bears the responsibility of harmonising its guidelines with the international community, it is important that the guidelines are well-balanced with respect to the realities of the Canadian scientific community and the ethos of Canadian society. In addition, the Guidelines Development Program must ensure that its guidelines are based on sound scientific evidence. The CCAC *guidelines on: transgenic animals* (CCAC 1997) and the CCAC *guidelines on: the care and use of fish in research, teaching and testing* (in preparation) provide two examples of situations where sound scientific evidence is lacking.

In line with the principles of the Three Rs (Russell & Burch 1959), the CCAC has adopted an approach, throughout all of its guidelines, which emphasises the importance of minimising the potential for pain and distress for individual animals. In this context, a precautionary approach has been taken for both of these guidelines documents. The CCAC *guidelines on: transgenic animals* (CCAC 1997) recognises that GM animals may experience pain and distress as a result of their genetic modification, and therefore an evaluation is required before changing the category of invasiveness of the protocol to ensure that the animals receive adequate care and attention. The CCAC *guidelines on: the care and use of fish in research, teaching and testing* (in preparation) takes as its premise the fact that fish have the potential to experience pain and distress, and builds on this foundation to ensure that fish receive the same level of care, monitoring and treatment accorded to any sentient laboratory animal.

In order to provide an ethical framework for the use of animals in research, teaching and testing, guidelines need to be based on sound scientific evidence. However, when the evidence does not yet exist we have a choice, either to wait and maybe attempt to facilitate research that will provide the data on which to base our guideline statements, or to adopt a more proactive route and attempt to balance what is currently known with expert advice. This paper uses two case studies to chart a course for guidelines' development aimed at moving ahead with a precautionary approach. In

both instances, the end result is the provision of a useful tool for investigators and institutional animal care committees. More rapid implementation of guidelines in these areas should lead to improvements in housing and husbandry, as well as in the choice of procedures which minimise pain and distress for animals used in Canadian science.

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