AATEX **14**, Special Issue, 715-720
Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences
August 21-25, 2007, Tokyo, Japan

Categories of invasiveness – A precautionary approach

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Abstract
The Canadian Council on Animal Care (CCAC) oversight of animal care and use requires experimental protocols to be classified according to the invasiveness of procedures. The *CCAC policy statement on categories of invasiveness in animal experiments* (CCAC,1991) provides the basis for this classification and defines five categories A to E (from least to most invasive). These categories are further refined and explained through development of CCAC guidelines aimed at minimizing pain and distress. The CCAC has been able to monitor progress in the minimization of pain and distress through collation and publication of detailed national animal use statistics on the numbers of animals used within each of the categories of invasiveness. In order to provide accurate data, the CCAC also provides guidance on the submission of animal use data, for example through an interpretation bulletin posted on the CCAC website.

A number of factors related to the assignment of categories of invasiveness (CIs) in the Canadian system have an impact on the national statistics, and on comparisons with animal use data from other jurisdictions. These include prospective assignment of severity, and precautionary measures such as assigning the generation of all genetically-engineered animals to CI D, a severe category of invasiveness. These factors will be discussed with a view to sharing experiences in animal use data collation, and enhancing public accountability.

Keywords: categories of invasiveness, protocol review, Canadian Council on Animal Care, breeding colonies, genetically-engineered animals

Introduction

Categories of invasiveness (CI) alert investigators, animal care committees (ACCs) and those responsible for animal care to procedures where there is a risk of animals being exposed to pain and distress. The annual publication of animal use numbers per CI and per purpose of animal use (PAU) by the Canadian Council on Animal Care (CACC) provides a window for the public into the reasons for the use of animals in science, and the potential pain and distress experienced by those animals.

National policies can impact local decisions and affect data trends in ways that may not have been anticipated. The categorization of animal-based studies according to their potential to cause pain and distress to the animals has been in place in Canada since 1987. However, revision of this system is necessary for it to continue to be a useful tool, particularly in areas of emerging science. The generation of genetically-engineered animals raises particular challenges for this system. Prediction of the potential pain and distress for these animals is difficult, as the phenotype is often unknown until the gene of interest has been successfully inserted or silenced. For this reason, implementation of the CCAC *guidelines on: transgenic animals* (1997) requires that all protocols involving the generation of genetically-engineered animals be assigned to CI D, identified as having the potential to experience pain and distress. Unfortunately, this has had the unintended effect of inflating the numbers of animals in CI D (potential to experience pain and distress). Through the current revision of the CCAC *guidelines on: transgenic animals* (1997) the assignment of CIs for these types of protocols, is being re-examined both at the local level, and at the national level. As part of this revision, consideration is being given to guidelines and polices that are emerging from other countries, with the aim of enabling information on the well-being of the animals to be shared between users, and reported in a manner that permits international comparisons.

Canadian Council on Animal Care

The CCAC is the national organization with the responsibility for overseeing the care and use of animals in Canadian science (http://www.ccac.ca). Through the overarching *CCAC policy statement on: ethics of animal experimentation* (CCAC, 1989), the CCAC has incorporated adherence to the Three Rs principles of Russell and Burch (1959) as the fundamental basis for the ethical oversight of animal care and use in Canada.

The CCAC delivers its mandate through three interrelated programs: Assessment, Guidelines and Education Training and Communications, which operate through an evidenced-based learning loop model (Gauthier & Griffin, 2005). In addition, the CCAC's ethical review and surveillance system is designed to ensure quality control at the local level by integrating the needs of scientists, animals and the community through institutional animal care committees (CCAC, 2006a), and to provide quality assurance at the national level by setting and ensuring the consistent and universal implementation of its standards for the care and use of animals in science.

Protocol review

The CCAC requires that all institutions conducting animal based research, teaching or testing establish and maintain a functionally active ACC that reports to the senior administration of the institution. Membership of an ACC includes:

- scientists and/or teachers experienced in animal care and use, who may or may not be actively using animals during their term on the ACC;
- a veterinarian experienced in experimental animal care and use;
- an institutional member whose activities, past or present, do not depend on or involve animal use for research, teaching or testing;
- a student representative at teaching institutions;
- at least one, and preferably two or more individuals representing community interests and concerns, who have had no affiliation with the institution, and who have not been involved in animal use for research, teaching or testing; and
- the ACC coordinator (ie the institutional employee who provides support to the ACC).

In addition to the individuals listed above, the person with overall responsibility for the animal facilities, whether a veterinarian, a scientist or a technical staff member, must be included on the ACC. ACCs also may include occupational health and safety and biosafety representatives (if this is not the case, other ways must be found of ensuring close links), as well as biostatisticians, ethicists and those responsible for public relations.

The operation of each ACC must be governed by formal Terms of Reference these include the Terms of Reference provided by the CCAC (CCAC, 2006a), but need not be limited to them. The ACC's Terms of Reference should be tailored to the institution's animal care and use program, including the members of the program and the institution's policies, practices and procedures.

One of the key responsibilities of ACCs is the ethical review of any animal-based studies to be carried out within the institution or by investigators or study directors employed by the institution (CCAC, 2003a). No animal-based work is permitted to start until approval has been received from the ACC. Elements that are considered during the ethical review of a protocol are outlined in the *CCAC guidelines on: animal use protocol review* (CCAC, 1997a). These include a focus on implementation of the Three Rs, such as justification for the selection of an animal model, details of the experimental design; species-specific housing and husbandry requirements and experimental procedures that are the least invasive for the animals. The ACC is responsible for ensuring that all animal-based protocols comply with CCAC guidelines and policies, and, if at variance with those standards, that justification for the variance on scientific grounds is provided.

Categories of invasiveness

The assignment of categories of invasiveness to protocols signals to investigators, veterinarians, animal care staff and ACC members the protocols which should be given particular attention due to their potential to cause pain and/or distress to the animals.

Investigators using animals are required to complete a protocol review form, to facilitate review of the study by an ACC. One of the elements of the form is the assignment of a CI to the study. If the ACC determines that the CI has been inappropriately assigned, it may be adjusted before the study begins. The CI may also be adjusted by the ACC after the work has begun, usually following a one-year report on the work to the ACC. In assigning the CI, investigators and ACCs use the *CCAC policy statement on: categories of invasiveness* (CCAC, 1991). Additional information, including examples of CIs for particular types of studies, is also available on the CCAC website and in CCAC guidelines documents. For example, the *CCAC guidelines on: the care and use of wildlife* (CCAC, 2003b) provides a list of wildlife studies assigned to various CIs as an appendix.

The assignment of CIs is based on a **precautionary** approach according to the **potential** level of pain and

distress that animals **might** experience. There are five Categories of Invasiveness:

A = Experiments on most invertebrates or on live isolates
B = Experiments which cause little or no discomfort or stress
C = Experiments which cause minor stress or pain of short duration
D = Experiments which cause moderate to severe distress or discomfort
E = Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals.

The following gives examples of the level of invasiveness assigned to particular procedures:

- protocols involving the use of tissue, tissue culture, eggs, invertebrates, protozoa or other animal use where neither vertebrates nor higher invertebrates are held captive or affected should be assigned to CI A;
- protocols that involve **removing less than 0.5mm of tissue from the tip of the tail** of an animal to identify its genotype should be assigned a level B of invasiveness, provided that it is carried out between the ages of 3-4 weeks;
- protocols involving **oral gavage** (tube feeding) should be assigned a level C of invasiveness;
- protocols involving the creation of a **transgenic animal** should be assigned a level D of invasiveness as currently stated in Section 1b of the CCAC *guidelines on: transgenic animals* (1997b). Once the transgenic animal is created, the CI assigned depends on the resulting phenotype and on the nature of procedures to be conducted on the animal;
- protocols involving **electrofishing** should be assigned a level D of invasiveness, although the CCAC encourages institutions to use alternatives to electrofishing; and
- protocols where death is the endpoint and where the animals may experience pain and distress that cannot be alleviated, such as the mouse bioassay for shellfish toxin testing, should be assigned a level E of invasiveness.

The concept of a scale to rank painful procedures arose from Smyth (1978) as a basis for replacing animal experiments, with more effort to be placed on the replacement of those procedures which are the most controversial because they cause substantial pain to the animal. Several countries built on this concept and adopted various systems of pain categorization. The system used by the CCAC arose from a 5-point scale developed by Orlans (1987) and presented at the first conference organized by the Scientists Center for Animal Welfare. A detailed history of the development of pain scales internationally is provided by Orlans (1993).

The pain scales that have been developed fall into two main types: those that use "severity bands", for example the UK; and those that categorize *procedures* carried out on the animals. There appears to be a subtle but important difference between these two approaches. The first approach permits the assignment of a project to a severity band which includes the conditions under which the animal is to be housed, and the normal state of an animal (eg. colonies of diabetic rats might warrant a greater severity banding than rats not carrying a harmful mutation). The second approach, being procedure based, focuses on assigning a CI according to the likely experience of an animal to a particular procedure. This approach does not consider that an animal that might experience pain or distress as a result of its inherent genetic makeup and it assumes that the normal conditions under which animals are housed and cared for do not play a role in the pain and/or distress they experience.

Animal use data as a public policy tool

The CCAC has been publishing national surveys of animal use in science since 1975, which include both the purpose of animal use and the numbers of animals used by species. This is one of the primary ways in which the CCAC is accountable to the Canadian public. Since 1996, the CCAC has collected institutional annual animal use data in the format of the CCAC Animal Use Data Form (AUDF), in order to publish the annual *CCAC Survey of Animal Use* and to gather information prior to a CCAC assessment visit to an institution. The AUDF allows the CCAC to publish aggregate information on animal use in science without identifying individual institutions or animal users. It provides not only the numbers of animals used per species, but also the purpose of animal use (PAU) and the CIs for the procedures used on the animal[1]. The PAU is divided into the following categories:

- breeding colonies (PAU 0)[2];
- fundamental studies (PAU 1);
- medical studies, including veterinary medicine (PAU 2);
- regulatory testing (PAU 3);
- development of products (PAU 4); and
- educational purposes (PAU 5).

At the institution level, the ACC is responsible for collating and submitting animal use data to the CCAC in an appropriate format, as explained in the *CCAC interpretation bulletin on: the animal use data form* (CCAC, 2006b). For protocols, where there are

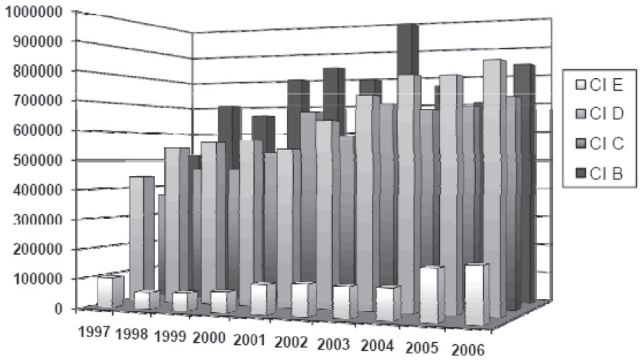

Fig. 1. Number of animals used per category of invasiveness (CI) from 1997 to 2006

The number of animals (x axis) is shown for each year between 1997 and 2006. For each year (y- axis) the numbers of animals used in each category of invasiveness are depicted. CI B is the least invasive and CI E the most invasive category relating to the potential pain and distress which may have been experienced by those animals as a result of the scientific procedures carried out on them.

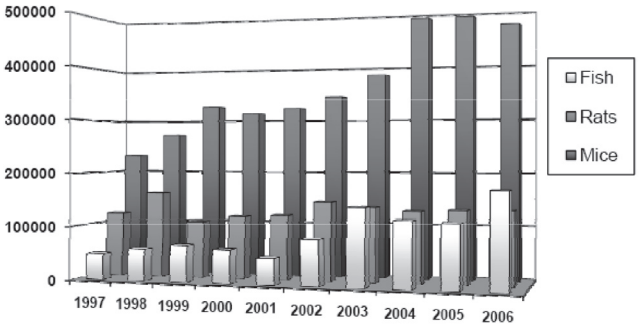

Fig. 2. Numbers of mice rats and fish used in CI D from 1997 to 2006

The number of animals assigned to CI D level of invasiveness rose by approximately 400,000 between 1997 and 2006. This was due to an increase in the number of mice and the number of fish assigned to this category. Mice and fish are the most common animals subject to genetic engineering. As the current CCAC guidelines require all protocols involving the generation of genetically-engineered animals to be assigned to CI D, it is probable that this increase in CI D was due in large part to the increasing generation of genetically-engineered animals from 1997 to 2006.

multiple levels of invasiveness, these can be reported separately with each animal assigned to one CI. Where there is re-use of an animal in the same year, the use is to be reported under the highest CI.

Prospective assignment of severity

Fig. 1 shows total numbers of animals used on an annual basis by Category of Invasiveness from 1997-2006. Currently, animal use is reported to CCAC prospectively; ACCs collate the numbers of animals that have been approved for use under a particular protocol number and submit this data to the CCAC. ACCs track the number of animals that have actually been used, but this number is not always included in the AUDF. In addition, CIs which are assigned when the protocol is reviewed may not be appropriate, as the outcome of the work and the actual level of pain and distress to be experienced by the animal is unknown at that point in time.

While the number of animals used has tended to increase since 1997 (from 1.75 million to 2.54 million) it is also apparent that the numbers of animals assigned to CI D has also increased (by approximately 400,000). We are ascribing this increase in CI D to the increase in creation of genetically-engineered animals, based on a similar increase observed in the use of mice (see Fig. 2) and on the trends reported by Ormandy et al (2008) in an analysis of the use of genetically-engineered animals in the literature. With the publication of the *CCAC guidelines on: transgenic animals* in 1997, the CCAC has applied a precautionary approach to protocols for the generation of genetically-engineered animals, requiring that they be assigned to a CI D. This CI designation can be revised once the phenotype of the animal has been shown not to have a negative impact on the welfare of the animal, however, this prospective reporting of animal use data may be overestimating the numbers of animals reported to be experiencing considerable pain and distress.

What to do with genetically-engineered animals?

The revision of the *CCAC guidelines on: transgenic animals* (CCAC, 1997b) as the *CCAC guidelines on: genetically-engineered animals* (in prep) is providing an opportunity to re-evaluate how this data might best be collected and reported to the public. We are currently developing an approach that will require the use of a three protocol system. A protocol will be required to generate a new animal line and this will be assigned to CI D. Once the impact of the genetic modification on the welfare of the animals is understood through careful monitoring of the welfare of the animals, probably over at least two generations, the new line may be transferred to a breeding protocol. This is the approach currently recommended in the *Australian National Health and Medical Research Council guidelines for the generation, breeding, care and use of genetically modified and cloned animals for scientific purposes* (NHMRC, 2007), where the local Animal Ethics Committee is responsible for approving the acceptability of transferring the new line to a breeding protocol. As a third step in this system, genetically-engineered animals that are used in experimental procedures, whether for phenotyping, or for other types of studies, will be assigned to a separate animal use protocol. The CI assigned to these protocols will depend not only on the invasiveness of the procedure, but also on the level of pain and distress already being experienced by the animal as a result of the genetic modification.

Categories of invasiveness for breeding colonies

At the present time, breeding colonies are assigned to a CI B, although this information is currently submitted to the CCAC on a voluntary basis only, and is not published in the annual statistics on animal use. As noted above, ascribing a CI to a protocol is intended to signal the amount of pain and distress that might be experienced by an animal, as the result of an experimental procedure. Causing animals to be born for research purposes, and in particular producing animals that will experience pain and distress as a result of their genetic mutation, is not ethically neutral. This dilemma has been addressed by the UK Home Office by labeling the breeding of animals with a harmful mutation an experimental procedure (Home Office, 2000). Considering the maintenance of a breeding colony as an experimental procedure, with a CI based on the level of pain and distress experienced by the animals as a result of their genetic make-up, would be new for the CCAC. However, it may be the most appropriate route to ensure careful welfare assessment of any newly created animal line, and appropriate designation for the animal line once it is transferred to a breeding colony protocol.

Conclusion

The system of assigning CIs to animal use protocols used by the CCAC since 1987 has been useful in signaling to investigators, ACCs, and animal care staff which protocols require the most attention to ensure that animal pain and distress is minimized. It has also been used as means of reporting to the public, in a generic manner, the levels of pain and distress that could potentially be experienced by animals used in science.

Reporting of animal use data is one way for the CCAC to remain accountable to the Canadian public in overseeing the use of animals in science. Additionally, collection of this information assists the CCAC in focusing on emerging trends and developing policy tools where needed. Through examination of animal use data, it became clear that the escalation in the numbers of protocols assigned to CI D, with a concomitant increase in the numbers of mice being used, was a result of the increasing generation of genetically-engineered animals. This signaled the need to revise the *CCAC guidelines on: transgenic animals* (CCAC, 1997b), in particular to address issues related to the management of breeding colonies, procedures for the generation of genetically-engineered animals, and the maintenance of breeding colonies.

During the revision of these guidelines, the CCAC has also begun to address the question of how to assign the correct CI to breeding colonies of animals. The vast majority of animals in breeding colonies do not experience pain and distress; this includes those that are genetically-engineered, most of which are not welfare-compromised.

However, it seems appropriate to recognize breeding colonies where animals are experiencing pain and distress as a result of the genetic modification. Assignment of the appropriate CI to these animal lines will serve the dual purpose of signaling, at a local level, that particular attention needs to be given to the care of those animals and, at the national level, where additional policy or guidance is needed.

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Footnotes

[1] Protocols assigned CI A are not included in the CCAC *Survey of Animal Use*

[2] Currently it is not mandatory to submit numbers of animals in breeding colonies to CCAC, and these animals are not included in the annual animal use data published on the CCAC website.