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Laboratory animal welfare: status and resources

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1 Introduction

There is a diversity of opinion in society regarding the use of animals in research. However, there is consensus that the welfare of research animals is a moral imperative and is critical to producing high quality science. The institutional "culture" has a profound impact on the level of attention to quality science and animal welfare. Klein and Bayne (2007) propose that the establishment of a culture of care, conscience and responsibility promotes an environment of workplace integrity, ethics-based decision making, good communication of institutional expectations, clear lines of authority, and a system for continuous development and improvement of the animal care and use program. When this institutional culture is established, institutional personnel will ground their decisions and actions in an ethical context that will promote quality science and animal welfare. However, challenges still exist in terms of assessing individual animal welfare for some species commonly used in research, particularly for rodents. Efforts to develop score sheets that accurately reflect the welfare status of the animal have been productive, as has the application of newer technologies to record physiological data about the animal which inform an extrapolation to welfare assessment. As a result, housing environments have been improved, intervention points are better defined, and refinements in experimental procedures have been implemented to support the research animal's welfare.

2 Defining Animal Welfare

Regardless of the method of research animal oversight, steps to ensure research animal welfare must be framed by a sound understanding of what good welfare is, and a means to verify that the animal is experiencing good welfare. There are numerous definitions for animal welfare that may be applicable to the specific case of research animals. The World Organisation for Animal Health (OIE) has recently published standards for care and use of animals used in research, testing or education (http://web.oie.int/eng/normes/mcode/en_chapitre_1.7.8.htm) that reference the OIE's definition for animal welfare. These standards are part of the broader Terrestrial Animal Code published by the OIE. The OIE is comprised of 178 countries and territories (http://www.oie.int/index.php?L=3&id=103) who pledge to follow the standards in the OIE's Codes. Therefore, these standards have far-reaching influence. The OIE states that animal welfare: "means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress…. Animal welfare refers to the state of the

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animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment."

The American College of Animal Welfare (www.acaw.org), an organization comprised of veterinarians with special expertise in animal welfare, states that "Assessment of welfare includes consideration of the animal's health, behavior, and biological function." A combined approach to using the OIE definition and ACAW's criteria for assessing welfare offers staff at research institutions is a credible means of ensuring laboratory animal welfare that is based on fundamental principles of good health, good husbandry, minimizing pain and distress, and opportunities to express species-appropriate behavior.

3 Assessing Research Animal Welfare

Interest in developing reliable methods of assessing laboratory animal welfare has increased in recent years. For example, the National Institutes of Health, one of the largest research organizations in the world, has an AnimalWell-Being Interest Group for the intramural research program. Also, there is a research animal focused Animal Welfare Group on Linked-In that is quite active and covers a wide range of welfare topics. Organizations such as Public Responsibility in Medicine and Research (PRIM&R) and the National Centre for the Three Rs (NC3Rs) have included this topic in conference programs, and in the case of the NC3Rs, in cooperation with the Biotechnology and Biological Sciences Research Council, proposals for research into welfare assessment are solicited for grant opportunities. Topics for research grant proposals include: pain assessment in animals; the development of validated behavioral, physiological, and cognitive indicators of welfare; the development of validated measures of affective states in animals; the development of accurate welfare measurement tools that can be used under lab and/or field conditions; and the development of novel methods for the assessment of cumulative suffering and severity.

Hawkins (2011) have noted that there are several key elements to an effective program for assessing research animal welfare. Typically, such assessments require a team approach constituted of animal care personnel, research personnel, the veterinarian, and the Institutional Animal Care and Use Committee (IACUC). Each member of the team brings a different perspective to animal observations. For example, an animal caretaker sees the research colony daily and thus is often most sensitive to nuanced changes in an animal's behavior. These staff members see and work with the animals in their home cages, where animals might display different behaviors from those a research technician might see in the experimental situation. The veterinarian may be focused on how the physical health of the animal is impacting its overall welfare, and the IACUC members who inspect the animal facility and observe the animals in the context of the entire animal care and use program may be especially attentive to how an animal's use in a research project conforms with the approved protocol and if there have been any unexpected welfare consequences. These different types of observations are complementary and are important to developing a composite picture of the animal's health and welfare.

Although approximately 90% of animals used in research are rodents, the range of species and strains of animals used in research is very broad. Thus, it is critical that the parameters used to assess welfare are customized to the species, strain, age of animal, as well as the type of research use. This necessitates sound training programs for personnel making these assessments, with some method of validating the accuracy of the assessment procedure and results. This process begins with establishing criteria for defining good welfare in the species/strain of animal, but also takes into account any clinical and/or behavioral signs that may be the result of the experiment. As a corollary, intervention points or humane endpoints should preferably be identified before animal use is initiated, for example during the development, review and approval of the animal use protocol. A system of timely communication of any welfare concerns is essential. Such a system should have clear reporting lines and typically encompassesafter-hours monitoring

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and a notification system to ensure prompt attention to issues that may arise.

3.1 Methods of welfare assessment

There are two primary methods for conducting welfare assessments: 1) use of subjective observations and judgments (e. g., skin color of neonatal mouse pups (Wells et al. 2006)); and 2) use of objective score sheets that have specific criteria to evaluate (e. g., the number of mice in or out of the nest (van der Meer et al. 2001)) for which the response is either yes/no, or a number ranking. The optimum welfare assessment is sufficiently broad to include a variety of indicators of good health and normal behavior. For example, the score sheet advocated by the New Zealand government, which is based on a system developed by Dr. David Morton (University of Birmingham, UK), embraces this principle by including measures of body weight and animal responsiveness (bright, alert, responsive); general clinical signs (rough hair coat, dehydration, nasal discharge); behavioral signs of pain (evidenced in rats, for example, by belly press and arched back); daily water consumption; an evaluation of any surgical site; and post-operative support activities (NAEC 2010). The welfare assessment is most practical if it can be associated with routine husbandry procedures and is minimally or non-invasive (Wells et al. 2006).

The Canadian Council on Animal Care (CCAC) has proposed three primary sources of information to aid staff at research facilities in making welfare assessments (http://threers.ccac.ca/en/alternatives/refinement_raffinement/theme14.html). They are:

1. Information from routine colony management such as longevity, growth rate, susceptibility to disease, reproduction and infant care, wound healing, coat and body condition, body shape and posture.

2. Information from structured behavioral assessment such as documenting the animals' behavioral repertoire and activity budgets (including grooming, sleeping, play, social behaviors, facial expressions and vocalizations).

3. Information from instrumentation such as heart rate, blood pressure, body temperature, serum levels of stress hormones (e. g., cortisol), and immunological functions such as rates of lymphocyte proliferation and suppression of lymphocyte activity.

The CCAC acknowledges that each of these sources may have limitations. For example, information derived from instrumentation can require an invasive procedure to place thedata-collecting equipment in or on the animal, or the animal may be handled more to obtain the data (e. g., removal of the animal from its cage and placement on a scale to obtain body weight information). The CCAC also recognizes that the quality of the behavioral assessments depends in large part on the training and skill of the observer. In some cases animals will not exhibit signs of pain in the presence of human observers (personal observation), or changes in behavior that indicate a decline in welfare may initially be quite subtle and require skill in detecting at an early stage.

3.2 Ensuring observer accuracy

The potential for errors in the interpretation of behaviors being introduced due to inadequately trained observers or observer variability is widely recognized as being a potentially limiting factor in enhancing the welfare of laboratory animals. If an indicator of poor welfare is missed and the animal's welfare continues to decline, then the inciting cause of the reduced welfare goes unchecked. If a behavior is misconstrued as signaling declining welfare, an intervention may be inappropriate. A significant aid in this regard is the series of video tutorials available through Newcastle University's Assessing the Health and Welfare of Laboratory Animals (AHWLA) website (http://www.ahwla.org.uk/site/Tutorials.html). The "Users Guide" to the website states that the goal is "to provide practical guidance in recognising signs of health and good welfare and to help users of the site to become better able to identify signs of pain, distress and poor welfare in laboratory animals." More timely intervention and an ability to determine if refinements to procedures are having a meaningful impact on the welfare of the animals naturally follow from this training. The tutorials take basic measures, such as coat condition or recognizing behaviors associated with

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pain, and use photographs or videos to demonstrate the difference between good welfare and poor welfare. The tutorial is constructed in a question/answer format, with explanations for animal conditions (illustrated with photographs) addressing the full range of possibilities. For example, photographs are provided of an under-sized piglet and mouse. The explanation provided for their condition includes the normal circumstance that some animals are born small to etiologies such as malnutrition, dental abnormalities, disease or genetics. The tutorial uses several readily observable parameters to illustrate acceptable and poor welfare. These include: body condition, changes in body shape, posture, fur and feathers, facial expression, skin, mucous membranes, eyes, ears, nose, mouth, tail and perineum. Acceptable handling and restraint techniques for a variety of animals are also demonstrated with photographs.

Also available on-line at no cost is a series of photographs and video clips of various species of animals (including several laboratory animals) exhibiting stereotypic behavior. Dr. Georgia Mason (University of Guelph, Canada) has established this video and image library (http://www.aps.uoguelph.ca/~gmason/StereotypicAnimalBehaviour/library.shtml) as a teaching tool to aid in the recognition of repetitive abnormal behavior in its various manifestations. She notes that in some species, such as laboratory rodents, animals display their stereotypic behavior at night or during the dark cycle of facility operations when personnel are typically not present. This serves as an important reminder that welfare assessments of laboratory animals may need to be recorded after routine work hours to obtain accurate information about the status of the animals.

3.3 Scoring Systems

The use of key physical, physiological or behavioral characteristics as a basis of welfare judgments has been recommended for some time. In some cases, subjective evaluations are made regarding the animal's status. For example, the National Research Council (1998) suggests that the welfare of nonhuman primates used in research be judged based on four behavioral metrics: 1) the animal's ability to "cope effectively" with changes in the social and physical environment; 2) the animal's ability to engage in "beneficial" species-typical activities; 3) the animal should not be expressing maladaptive or pathological behavior that results in injury or other "undesirable consequences"; and 4) the animal should manifest a "balanced temperament" with no chronic signs of distress. The authors of this NRC report note that the animal should express behavioral diversity (i.e., have a relatively broad behavioral repertoire) and that some kinds of behaviors may be more practical indicators of good welfare than others (e.g., while reproduction is generally considered to be a relatively sensitive indicator of welfare status, not all laboratory primates are allowed to breed, thereby rendering this measure of little to no value in most research facilities). The report underscores the importance of determining the etiology of atypical behavior patterns before initiating an intervention strategy to ensure the problem is correctly addressed. While this guidance is valuable, it is fairly general, and leaves much to interpretation. Bayne and Novak (1998) published a behavioral assessment scale for abnormal behaviors in rhesus monkeys. The scale ranges from 1 ~ 6 and is based on atypical behaviors expressed that are generally considered non-detrimental to the animal (e.g., circling in the cage) at the low end of the scale to behaviors that are uniformly considered harmful to the animal (e.g., self-injurious behavior such as self-biting) at the high end of the scale. The goal of such a scale is to assist decision makers in judging the severity of the animal's condition and thus its welfare status, its potential to recover from the pathological condition, and its value as a research subject.

The obvious benefit to the use of score sheets when assessing research animal welfare is the objectivity they can introduce to the process. However, it is important that the individual conducting the assessment is well trained and competent at identifying often subtle signs of reduced welfare and that personal bias is eliminated from the process. Thus, familiarity with signs of reduced welfare and reliability in

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classifying the observation in a severity scale are skills that must be cultivated in the observer(s). If more than one observer is conducting the assessments, inter-observer reliability should be verified. Score sheets in which the majority of observations recorded reflect objective information (e. g., body weight, size of tumors, etc.) facilitate reliability of observations within and across observers. One example of this method is the scoring procedure developed by Honess et al. (2005) to assess the degree of alopecia (hair loss) in nonhuman primates. Alopecia in nonhuman primates is a complex problem which is generally considered to have multiple etiologies (Novak & Meyer 2009). Causes may be natural and expected (e. g., seasonal, aging) or reflect poor welfare (e. g., hormonal imbalance, bacterial or parasitic infection, nutritional deficiency, etc.). Honess and colleagues (2005) developed an objective scale that denotes the size of the affected area either as a percent of the skin surface (e. g., the portion of the back of the animal that is affected) or for smaller patches of hair loss, in square centimeters. However, not all scoring systems are this objective. Rather, many pivotal assessment criteria are more subjective, such as the interpretation of an animal's facial expression, or require training and experience to competently judge the measure, such as behavior recordings and coat condition. With training and practice, these assessment criteria can become quite reliable indicators of good or poor welfare.

The welfare of transgenic mice is of particular concern due to the sometimes unpredictable phenotypic results with a new transgenic line, and because there are increasing numbers of transgenic mice used in research. Van der Meer et al. (2001) have published a score sheet for different age ranges of transgenic mice that relies primarily on objective measures that are sufficiently straightforward to be reliable across observers. For example, for newborn mice day 0 (day of birth) through day 6, the observer records if the mouse is in or out of the nest and the number of pups with a visible milk spot (milk present in the stomach). These notations are also made for mice day 10 through day 14, as well as fur growth, tooth eruption and whether the ears and eyes are open, as well as if the pup is walking and righting itself. At weaning and after weaning, the posture of the pups (normal or huddled), and reaction to opening the cage and being handled (hyperactive, fear, aggression) are recorded. The objectivity of this scoring system makes it particularly appealing; however, the authors note that such observations in a large mouse colony are very time consuming, adding perhaps 15 ~ 20 minutes to the husbandry time for a litter of 4 ~ 6 pups.

This illustrates the trade-off, and challenge, of scoring systems that promote an objective assessment of the welfare of individual animals, but are constrained by large numbers of animals in the research colony. Wells et al. (2006) attempted to address this paradox by recommending an assessment scheme that requires a minimal investment of additional time or additional burden of paperwork. They suggest that welfare assessments initially take into account any known strain characteristics (e. g., noise-induced seizures) as a baseline upon which additional genetic alterations are overlaid. The assessment can be made at key points in the animal's life, such as when the mouse is a neonate, at weaning, and at relevant time points when the animal is an adult. When a new line is established, these assessments are particularly important, as they serve as the benchmark for predicting welfare changes in other animals of the same line. In this manner a welfare profile is developed for the various genetic lines used at the institution.

3.4 Assessing the welfare of animals in cancer research

Perhaps the most well-known guidance regarding humane endpoints and other considerations related to the welfare of animals used in cancer research (which are principally mice) have been published under the sponsorship of the United Kingdom Coordinating Committee for Cancer Research (Workman et al. 1988 and 1998). These guidelines have recently been updated (Workman et al. 2010) to reflect increased emphasis on the Three Rs and new assessment technologies. The authors note that early endpoints are preferred in cancer research to minimize "non-specific

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systemic effects," thereby fostering a greater correlation between the results obtained and the tumor effect. Workman and colleagues propose a battery of clinical signs that should lead the investigator or institutional veterinarian to immediate intervention. These signs include: absence of eating or drinking over a 24 ~ 48 hour period resulting in emancipation or dehydration, persistent hypothermia, labored respiration, hind limb paralysis or weakness, tumors that interfere with locomotion or cause abnormal vocalization, and others. They recommend that where any one sign is observed, the animal should immediately be euthanized and increased monitoring of the animal's cohorts initiated.

The latest guidelines state that the frequency of examination of animals will be determined in part by three factors: 1) the known biology of the tumor and/or the effects of the inducing agent; 2) the effects of any associated techniques; and 3) the changing clinical status of the animal. In general, the examination should be comprised of an assessment of the overall clinical condition of the animal, including its physical appearance, body posture, body temperature, behavior and physiological responses; observation of its food and water intake; obtaining body weight measures to determine changes in tumor mass or volume; and observation and palpation of the tumor sites to note any possible distension, ulceration or compromised locomotion of the animal. Workman et al. (2010) describe several new imaging technologies that can aid these assessments. These include optical surface bioluminescence of orthotopically grafted cancer cells transfected with luciferase, which is useful for "deep-seated" tumors; positron electron tomography (PET) imaging of tumor cell proliferation using ^{18}F-3′-fluoro-3′-deoxy-L-thymidine (FLT), one of several radiotracers useful for studying biochemical pathways *in vivo*; and intravital imaging of tumor vasculature using multi-photon fluorescence microscopy. Such non-invasive monitoring techniques facilitate monitoring of tumor progression and thus earlier humane endpoints for the study animals.

4 Challenges to overcome

A number of practical considerations present challenges that must be overcome for any research animal welfare assessment tool to be widely accepted and implemented. Perhaps the most significant of these is the sheer number of rodents that can be present at a single institution (tens of thousands in some cases) and the issue of conducting individual animal welfare assessments in such cases. Thus, the large colony size at a single institution can result in a balance being struck between a "herd health" approach and ensuring individual animal welfare. One strategy to address this dilemma is to triage certain strains or lines of rodents based on their vulnerability, thereby providing a more intense assessment approach to the more fragile animals. Yet, in many cases even this method is difficult due to the newer technologies in caging systems which have become commonly used, such as individually ventilated cage (IVC) systems, and can impede visualization of the animals through the sides of the cage. Often, the cage must be partially drawn out of the IVC rack to look down upon the rodents, which may result in disturbing them.

Although van der Meer et al. (2001) state that one challenge to welfare assessment is to select measurable biological parameters that reflect a variety of key criteria (physiological effects, immune suppression and behavioral responses), an equally significant obstacle to an assessment technique utilizing a variety of parameters is the level of staff time involved in making the welfare observations and completing related documentation. Also, once the data are collected it should be determined if the information will be stored in an electronic database, and thus who will enter the data and how the information will be used in the future (e. g., as part of a mouse passport). The economic constraints to establishing a welfare assessment system cannot be ignored; however, such constraints should not preclude institutions from evaluating various systems of welfare assessment and tailoring an approach to the animals used and type of research conducted. The minimization of pain and

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distress is a moral obligation and the welfare assessment tool is the foundation upon which husbandry, veterinary medical care, and humane endpoint decisions rest.

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