2009

# Addressing Distress and Pain in Animal Research: The Veterinary, Research, Societal, Regulatory and Ethical Contexts for Moving Forward

Kathleen Conlee
*The Humane Society of the United States*

Martin Stephens
*The Humane Society of the United States*

Andrew N. Rowan
*The Humane Society of the United States*

**Addressing Distress and Pain in Animal Research: The Veterinary, Research, Societal, Regulatory and Ethical Contexts for Moving Forward**

*Conlee, K.M., Stephens, M.L. and Rowan, A.N.*

It is widely agreed, at least in principle, that distress and pain in research animals should be minimized for the sake of the animals involved, the quality of the experimental results, and the support of the concerned public. Distress and pain not only compromise animal welfare but also can have biological effects that compromise experimental results (e.g., Carstens & Moberg, 2000; Institute for Laboratory Animal Research, 2008; Robertson, 2002). Additionally, given the public's concern for animal welfare, it is not surprising that public approval for animal research declines dramatically when the animals are subjected to increasing levels of distress and pain (e.g., The Humane Society of the United States, 2001). Clearly, distress and pain in research animals should be addressed for a variety of reasons, notwithstanding the associated difficulties and complexities.

While most people recognize that biomedical scientists are searching for knowledge that will improve the health of humans and animals, the image of someone deliberately causing harm to an animal in order to produce data that may lead to some future benefit has always prompted an uncomfortable reaction outside the laboratory. However, proponents of animal research have usually justified the practice by reference to greater benefits (new knowledge and medical treatments) over lesser costs (in animal suffering and death). Given that one of the costs of animal research is the suffering experienced by the animals, the goal of eliminating distress and pain in the animal laboratory wherever feasible, is one that few, if any, people (especially scientists) would argue against. Moreover, we contend that a laudable, long-range goal would be the elimination of all substantial distress and pain in the animal laboratory (Stephens and Conlee, 2004) although some might see this as an insurmountable technical challenge.

A prerequisite to effectively tackling the issue of animal distress and pain in research is a focused effort to define what is meant by these terms. This is not a trivial task despite being apparently straightforward. Robust definitions are key to determining how we can best tell when an animal is suffering significant pain and distress, how to measure this suffering, and what areas of research and what techniques cause such pain and distress. Aided by definitional clarity, one can then to look for alternatives that will allow science to progress without causing such harms to animals.

The Journal of the American Veterinary Medical Association (JAVMA) published a workshop report in which pain experts identified gaps in knowledge similar to those expressed here (Paul-Murphy et al., 2004); however the report focused only on pain. The report's recommendations included the creation of validated species-specific pain scales, support of a multidisciplinary approach to treating animal pain, creation of a special interest group within the International Association for the Study of Pain, improvements in funding for pain management research, and outreach to the public about animal pain. While the group did not address distress, they did discuss many other factors that are essential to addressing animal pain.

Collaboration among stakeholders is essential to tackling pain and distress issues. These stakeholders include policymakers and regulators, veterinarians, practicing scientists, animal protectionists, and other laboratory personnel (including caretakers and technicians). For example, the Joint Working Group on Refinement, established in the United Kingdom (Humane Society of the United States, 2003), which consists of various stakeholders, thus far has produced ten publications (one currently in preparation) focused on refinement in animal research and could serve as a model for a similar group in the United States.

This present book seeks to address many of these issues and to provide scientists and others with existing valuable information regarding animal pain and distress in the laboratory. The book focuses on distress, both pain-induced and non-pain induced. It is divided into two sections, with the first addressing concepts of animal distress, and the second, the causation, recognition, measurement and alleviation of distress.

Terminology

A discussion of terminology is a vital and necessary starting point. Despite this importance, terminology is often not tackled. For example, the recent ILAR report on “distress” did not discuss the range of states that might produce distress nor how those states differed from one another. The report did provide a useful review of the physiology of stress and distress and some indications of how the mental state of an animal might influence whether it was in distress or not but the lack of attention to terminology is a definite short-coming. (Institute for Laboratory Animal Research, 2008)

The terms "pain", "distress", "anxiety", "fear" and "suffering" describe experiences, and responses to experiences that are, in most cases, unpleasant and hence undesirable. Such terms are commonly used in everyday language to describe both human and animal experiences. However, the difficulty lies in understanding exactly what is meant when we actually use these terms. Dictionary definitions are often circular and unhelpful. For example, in the 1967 unabridged *Random House Dictionary*, pain is defined as both a sensation of acute physical hurt or discomfort and as emotional suffering and distress. Suffering is then defined as undergoing pain or distress. The *Random House* and other dictionaries appear to view pain, distress, and suffering as synonyms. However, a closer analysis reveals that this assumption is not supported.

The following are existing definitions of commonly used terms:

NOCICEPTION: The process whereby potentially noxious and/or tissue damaging
stimuli cause special receptors (nociceptors) to fire and send a nerve impulse along the nociceptive pathways. Pain perception may occur, but only when such nerve impulses are processed in the central nervous system. Pain perception is not a necessary part of nociception.

PAIN: An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (IASP, 1979). Pain terms are very variable and people may talk of acute or chronic pain, or sharp or dull pain, for example. Pain, as a focus of research and study, is neither solely physical nor psychological. For example, pain usually has a physical location (even when it is referred pain) in the organism but pain levels and tolerance are greatly influenced by the psychological state of the organism.

ANXIETY: An emotional state involving increased arousal and alertness prompted by an unknown danger that may be present in the immediate environment (Kitchen et al., 1987).Unlike pain, anxiety is a diffuse sensation that has no specific location in the body. Numerous effective anti-anxiety drugs have been discovered and studied in animal models and these drugs elicit the same behavioral modifications in both humans and animals.

FEAR: An emotional state involving increased arousal and alertness prompted by an experienced or known danger present in the immediate environment (Kitchen et al., 1987). Fear has a specific object, anxiety frequently does not.

MALAISE: A state commonly occurring in association with bacterial or viral disease. In recent years, this state has come to be described as consisting of “sickness behaviors” – see Gregory, 1998. The state is associated with the production of a variety of cytokines and can be elicited by injecting those cytokines into a healthy animal.

DISTRESS: A state in which the organism is unable to escape from acute stressors or adapt to an altered external or internal environment. In acute distress, the organism will try to escape but in chronic distress, the organism will commonly engage in maladaptive (e.g. learned helplessness) behaviors (cf. ILAR, 1992).

SUFFERING : A highly unpleasant emotional response usually associated with pain and/or distress. (Kitchen et al., 1987). The adjective "emotional" stresses the affective nature of suffering. Suffering involves a threat to the "person-hood" or self-concept of an individual rather than simply to the organic

body and is a metaphysical concept. It cannot be reduced to "operational" terms and is, thus, not easily incorporated into "objective" sciences.

The following figure (Figure 1) illustrates one model of how the above terms may be related. Affective states such as pain, anxiety, fear, discomfort are viewed as stimuli that can produce “distress” if they occur with sufficient intensity or endure for a long enough period of time. In this sense they can be viewed as “stressors” that may lead to the aversive state here identified as “Distress.” Suffering, in the model below, requires some cognitive processing. Thus, “suffering” can be seen as a “higher order” state that may not occur in beings with simpler nervous systems (e.g. crustacea). Even if suffering is absent, this would still not necessarily eliminate all human moral duties towards such beings.

Pain, fear, anxiety, discomfort and distress are all negative subjective states of being, and are typically described and grouped together under one larger heading of "suffering”. "Suffering" is a widely used and abused colloquial term that has been subjected to very little careful analysis, even in the case of human suffering. Cassell (1982), one of the few to address the biological and psychological roots of human suffering, argues that suffering occurs when the integrity of a person (not the body) is perceived to be compromised or threatened in some way. (Person-hood is defined in terms of an individual's mental life and is distinguished from the organic body). Damage to organic tissues can and often does lead to suffering but, for Cassell, it is the psychological reaction to such damage that is the key to understanding the idea of suffering.

The notion that suffering arises from a perceived threat to the integrity of a "person" has significant ramifications for any discussion of animal suffering. Animals would, according to the above definition, suffer only if they possess to some degree the qualities of person-hood. In a later analysis, in which he specifically addresses the issue of animal suffering, Cassell (1989) (and more recently Byrne, 1999) argues that only beings with a sense of the future (anticipation) and a sense of self are capable of experiencing suffering. Some animals do appear to have a sense of self (e.g. chimpanzees and other great apes) and a sense of the future or, at least, seem to be able to anticipate and reflect on future events. How far such abilities extend through the animal kingdom would necessitate a much more detailed analysis than is possible here. One could also argue that only animals that are capable of affective (e.g. emotional) responses might be included among the category of beings capable of suffering (Damasio, 1994).

It is quite clear that few, if any, people use suffering in the narrower sense articulated above - referring only to perceived threats to the "person" rather than simple vigilance to protect against threats to the non-reflective organism. Even scientists who object to using the term "suffering" when referring to animal distress will, nevertheless, still argue vehemently that animals (including invertebrates) are capable of suffering. However, the colloquial term "suffering" has such broad meaning that it cannot be used profitably (even after careful definition) when trying to assess the severity of aversive stimuli to animals, or even to discuss the level of distress experienced by animals.

In order to understand the underlying reasons for animal suffering and to alleviate its occurrence in laboratory animals, we must first examine its components. In the model presented in Figure 1, pain, fear, anxiety and discomfort are all aspects of the external, behavioral manifestation of underlying processes. For instance, a painful stimulus applied during an experimental procedure, given its intensity, duration and frequency of application, may lead to anxiety and fear. The animal comes to expect (predict) the arrival of the painful stimuli and therefore develops anxious and fearful reactions to any prior stimuli that are linked in time and space to the onset of pain. The sight of the hypodermic needle approaching, causing an animal to cringe, is one example. This cascade of cognitive-emotional responses can be termed 'distress'.

The cognitive-emotional filter through which an animal perceives its subjective experiences of the external world will in turn influence its internal states of being. If the animal perceives that the onset of pain is to be expected, perhaps on a daily or hourly schedule, it may suffer emotionally from the anticipation or expectation of the pain. In this case, the animal's emotional state and behavioral response may extend beyond its initial responses to the degree of pain inflicted by the original stimuli. Thus, the negative

emotional states experienced by the animal may not only contribute to but increase its sensitivity to the painful stimuli it anticipates.

The well-studied state of "learned helplessness", which occurs in both human and non-human animals, illustrates the point that cognitive-emotional suffering may be even more intolerable to an animal than the physical infliction of pain. Animals in states of severe suffering may display learned helplessness in which they typically show no response or attempts to withdraw or protect themselves from mildly painful or harmful stimuli. Humans who display learned helplessness are typically individuals who have been subjected to severe torture, physical, and mental/emotional abuse. This gives an idea of the perceived events that an animal has experienced to reach the state of learned helplessness. Animals exhibiting learned helplessness do not respond to therapeutic attempts to rescue their severely abnormal behavior and non-survival tendencies (Seligman, 1975).

**Human and Animal Pain and Distress**

Until recently, non-verbal human infants were considered by the medical community to be without the capacity to fully experience pain, highlighting the importance of verbal report in legitimating pain perception (Anand et al., 1987). This had practical consequences. In 1987 it was found that 85% of neonatal anesthesiologists agreed that human infants could experience pain but only 5% actually delivered pain relief. By 1996, 85% were giving pain relief (Rowan et al., 1998). Thus, ten years of focused scientific concern and efforts to draw attention to the issue of infant pain had a dramatic effect on the delivery of pain relief and consequently the very real experience of newborn infants. The fact that human neonates could not speak and describe their feelings was likely a contributing factor to the lack of appropriate attention to neonatal pain and suffering in the past. It is possible to study pain perception in animals using the same sort of techniques and reasoning by analogy as in human infants.

It has also been found that doctors tend to underestimate the level of pain experienced by their adult human patients (Marquie et al., 2003). If this is the case when the subjects are able to verbally express their level of pain, underestimation of animal pain is very probable. One research study looked specifically at this issue and confirmed the suspicion that animal pain and distress are not addressed as vigorously as they should be (Phillips, 1994). In this study, Phillips found, for example, that researchers seemed to have very little information regarding analgesics. The author witnessed cases in which animals were clearly experiencing pain due to waking up in the middle of invasive procedures (such as the drilling of holes in the skull), yet the researchers would continue the procedure without administering additional anesthetic. Also, in the majority of cases, researchers did not provide animals with analgesics following surgery. As a result, there is concern that a not insignificant amount of the pain and distress of non-verbal laboratory animals is overlooked and/or its severity discounted.

There is evidence, however, that attention to pain and distress has increased in recent years. For example, Richardson and Flecknell (2005) examined past and current anesthetic and analgesic use by sampling peer-reviewed articles that involved rats and mice used in one of six categories: skin incision, craniotomy, laparotomy, burn study, thoracotomy, or orthopedic. The sample included 238 articles published from 1990 to 1992 or 2000 to 2002. Of the full 238 articles, analgesic administration was specified in 20% of all papers from 2000 to 2002, but in only 3 % of papers published from 1990 to 1992. Overall, there was an increase in analgesic use and anesthetic regimens between the two time periods. For both periods, analgesics were most commonly administered for thoracotomy while few rodents undergoing laparotomy were given an analgesic.

While the apparent increase in anesthetic and analgesic use over time is promising, we would argue that specification of anesthetic and analgesic use in 20% of papers is an indication of largely insufficient attention to pain. As part of their examination, Richardson and Flecknell (2005) contacted Institutional Animal Care and Use Committees (IACUCs) and/or authors of 101 studies published from 2000 to 2002 that did not mention analgesia. Responses were received in regards to 28 of these papers. The majority of respondents (71%) indicated that they did not administer analgesics, 11% were uncertain, and 18% claimed that they administered analgesics but did not report it. Reasons given for withholding analgesics

included pain not observed, potential side-effects and interference with research protocols, and/or pain relief was thought to be unnecessary.

The Humane Society of the United States (HSUS) has also conducted analyses to gauge attention to pain and distress by the research community. One analysis found that the American Association of Laboratory Animal Science (AALAS) increased the number of sessions at their annual conference that addressed pain and distress issues (including pain, distress, humane endpoints, euthanasia, anesthesia, environmental enrichment and similar topics) from 8% of all sessions in 1998 to 21% in 2003 (The Humane Society of the United States, June 2004). Additionally, Plous (1996) determined that the number of scientific publications regarding animal welfare quadrupled following passage of the AWA in 1966. Despite this, there is still much work to be done in this area.

**The Research Context**

In addition to diminishing animal welfare, pain and distress can also negatively affect research results, which can have serious consequences. Pain and distress have physiological and neuroendocrine effects (Canadian Council on Animal Care, nd) and impact parameters related to respiration, heart rate, body temperature, and immunology, among others (American College of Veterinary Anesthesiologists, 2006; Page, 2002).

The impact of pain, distress and diminished welfare on research results can be found in the published literature. For example, the use of analgesics following surgery in rats with a form of mammary cancer reduced the spread of cancer in comparison to those who were not provided with analgesics (Robertson, 2002). Additionally, mice provisioned with environmental enrichment may mimic human disease, such as Huntington's disease, more closely than those not provided with enrichment (Hockly, Cordery, Woodman, Mahal, van Dellen, Blakemore, Lewis, Hannan, and Bates 2002).

Balcombe, Barnard and Sandusky (2004) conducted a review of eighty published studies regarding routine laboratory procedures, including handling, blood collection and orogastric gavage. Studies involving quantitative or behavioral measures of pain or stress during these routine procedures were chosen for examination. Handling, in this case, was defined as non-invasive manipulation for normal husbandry routines, such as lifting or moving an animal's cage. For the multiple species examined, the authors found significant changes in behavior and physiological parameters correlated with stress (such as heart rate, blood pressure, glucose, prolactin, corticosterone) in regards to all three procedures, with changes from baseline ranging from 20 to 100 percent and lasting 30 minutes or longer. Physiological response did vary with intensity of handling stressors. Overall, it was concluded that laboratory routines are associated with stress that is "quantified and substantial" and that the animals don't habituate to these procedures. The authors stated that "[t]hese data suggest that significant fear, stress, and possibly distress are predictable consequences of routine laboratory procedures, and that these phenomena have substantial scientific and humane implications for the use of animals in laboratory research." These findings only further support the argument that all sources of pain and distress, whether associated with research procedures, husbandry procedures, or living conditions should be addressed in order to minimize the resulting pain and stress.

Rowan (1990) similarly examined effects of common laboratory procedures, routines and housing on commonly measured biological parameters. It was noted, for example, that mice housed in conventional housing had significantly higher plasma corticosterone measures in comparison to mice housed in "low stress" housing. Rowan urged the use of means of assessing the impact of procedures, routines, and housing on animal behavior.

Clearly, pain and distress can impact the results of any experiments that involve the taking of physiological and behavioral measures, which includes the vast majority of animal studies. If this impact is serious enough, the ultimate consequence could be the confounding of any resulting clinical trials in humans or actual harm to the humans involved.

## The Societal Context

In principle, those who conduct animal research are ultimately accountable to the public, as such research is largely funded by public money, carried out for the public's benefit, and governed by public laws. Concern over research animal suffering helps shape the public's overall views on animal research. Most people supported animal research in the late 1940s, with very few disapproving (Herzog, Rowan and Kossow 2001). By the mid 1980s, however, opposition increased and remained steady at 30 to 40%, although this fluctuates according to various factors such as the species used, type of research, level of pain and/or suffering, and public trust (Herzog, Rowan and Kossow 2001). For example, in 1949, a small percentage of the public had concerns about specific species in research (e.g., dogs), but by 1983, 64 to 89% expressed concern for dogs, marine mammals, horses, birds, cats, farm animals, rabbits, and fish while a much lower percentage (twenty one to thirty four percent) expressed concern for rodents and reptiles (Doyle, Dane, Bernbach, Inc., 1983 as cited in Herzog, Rowan and Kossow, 2001). More recently, 52% of adults disagreed with the use of dogs and chimpanzees in research that produces new information about human health problems (National Science Board, 2002) (Figure 2).

Although many factors influence the public's support for animal experimentation, a key variable is the level of animal suffering, i.e., pain and distress. Support for research on all species declines when it involves pain or distress, yet this issue was largely unaddressed in opinion polls until the 1980s. From 1985 to 1996, opposition to research that caused pain and injury increased by 22% (National Science Board, 2002) (Figure 2). According to a poll conducted in the United Kingdom in 1999, approval of research on mice dropped by at least 18% when the research involved pain, illness, or surgery and dropped by at least twenty percent for monkeys (Aldhous et al. 1999) (Figure 3). A 2001 survey of Americans found that 60 to 75% disapprove of research involving moderate to severe pain and/or distress, respectively (Figure 4). One of the most recent surveys demonstrates that 76% of the British public believes that the government should prohibit experiments on any live animals that cause pain, suffering, distress, or lasting harm and people were equally opposed (80 to 90%) to the use of rats or mice being used in such research as they were cats, dogs, horses, monkeys, and rabbits (TNS Media, 2003 as cited in British Union for the Abolition of Vivisection, 2003).

There is evidence that members of the scientific community, including those who conduct research, have mixed feelings about animal research and are concerned about animal pain and distress. For example, Plous (1996) surveyed 3,982 randomly selected members of the American Psychological Association in order to assess attitudes toward the use of animals in psychological research and education. Overall, approximately 80% of respondents supported the use of animals in psychological research. This support dropped drastically, however, when animal pain or death was involved; only 17.7% "felt that painful or terminal experiments on primates were justified"; 18.8% felt this way with respect to dogs and 34% with respect to rats (Plous 1996).

Overall, opinion polls have demonstrated that public concern over animal research is steadily increasing; therefore the research community, particularly IACUCs, should begin to take concrete steps to substantively address these concerns.

## The Regulatory Context

Most of the countries that use substantial numbers of animals in research have laws or official codes of practice governing the welfare of the animals involved. The minimization of animal distress and pain is a primary (if not the overriding) aim of these mandates.

This is the case in the United States, where the principal law governing animal research practices is the Animal Welfare Act (AWA). The AWA was enacted in 1966 and amended several times since then. The 1985 amendments strengthened the AWA provisions on distress and pain (see Appendix A). These amendments specify that

- Pain and distress are to be minimized;
- Anesthetics, analgesics, and tranquilizing drugs are to be used, unless there is scientific justification otherwise;

- Alternatives to procedures that cause pain and distress are to be considered by the investigator (Appendix A provides the actual text of the legislation that relates to these issues); and
- Each registered institution form at least one Institutional Animal Care and Use Committee (IACUC) to review animal protocols and oversee the institution's animal care and use program.

The AWA grants authority to the USDA to create standards and regulations that pertain to the Act and to enforce these standards and regulations. Despite the fact that the term “distress” is used throughout the USDA regulations, the agency has never officially defined what is encompassed in its concept of distress. USDA Animal Care Policy #11 (Painful Procedures) defines a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied" (USDA, 1997). There is no comparable USDA policy that defines “distressful procedure,” even though distress can be pain induced or non-pain induced. To the agency’s credit, however, USDA Policy #11 does provide examples of procedures that may cause more than momentary or slight distress, such as food and water deprivation and paralysis or immobility.

The absence of a regulatory definition of “distress” means that inadequate guidance is available to institutions and may suggest inadvertently that the USDA is less concerned about distress than it is about pain. This could hamper or discourage institutions from expending the same effort in tackling distress as in addressing pain. The adoption and enforcement of a definition of distress would enable the research community to assess, prioritize and increase research regarding prevention, recognition, assessment and alleviation of distress; information that is recognized by the scientific community as needing clarification.

The USDA requires research facilities to submit an annual report of the number of regulated animal species used in procedures that fall into one of three categories. This official pain and distress classification system focuses heavily on whether or not animals were provided with pharmacological relief for pain and, to a lesser extent, distress (see Appendix B of this chapter). However, the system excludes—and therefore fails to encourage—many widely recognized non-pharmacological, yet highly practical means of reducing distress, such as by providing supplemental fluids, nutrition, or warmth (Hampshire, et al. 2000). Moreover, the system fails to reflect whether palliative measures were effective in reducing distress or pain. Consequently, the current classification system does not provide a clear picture as to what the animals actually experience with the unfortunate consequence that the system further obscures the issue of distress (particularly distress that is not caused by pain).

A lack of guidance on animal distress by the USDA could lead to inconsistent and inaccurate reporting of animal distress by institutions. Inaccurate reporting also has consequences, potentially leading to false portrayal of the costs of animal research to the public. Importantly, poor animal distress reporting could also prevent clear comparison of animal use statistics in the USA with those of other countries. Standardization of pain and distress issues is now deemed essential on the international level; for example, the Organization for Economic Cooperation and Development (OECD, 2000) guidance document on humane endpoints includes definitions of “distress” and other key terms.

Prior to 2002, the AWA language specified coverage of mammals and birds but the USDA chose to exclude all birds and lab-bred mice and rats from regulation. In 2002, Congress amended the AWA by essentially endorsing the USDA’s narrow definition of animal and specifically excluded birds, rats of the genus *Rattus*, and mice of the genus *Mus* who are bred for use in research from AWA coverage. Consequently, the AWA itself currently applies only to certain mammals. All institutions using any of these species in research must register with the USDA and comply with USDA standards.

In addition to the AWA, a second law also governs animal research in the U.S. The Health Research Extension Act (HREA) mandated that the National Institutes of Health (NIH) upgrade its requirements for animal research oversight. This bill includes a section on animal welfare provisions governing animal research. These provisions apply to all research facilities that receive funds from the NIH or its parent agency, the Public Health Service (PHS). The provisions were implemented through the PHS *Policy on*

*the Humane Care and Use of Laboratory Animals*, which deals largely with administrative procedures such as setting up an Institutional Animal Care and Use Committee (IACUC). PHS Policy is enforced by the NIH's Office of Laboratory Animal Welfare (OLAW) and applies to all vertebrate species, thereby partly compensating for the exclusion of birds, mice, and rats in the AWA.

PHS *Policy*, like the AWA, emphasizes avoidance or minimization of pain and distress (Appendix C). PHS Policy also incorporates the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training*; three of these nine principles directly address distress and pain (as detailed in Appendix C). Finally, PHS *Policy* calls upon research facilities to follow the provisions in the *Guide for the Care and Use of Laboratory Animals* (hereafter referred to as the *Guide*) published by the Institute for Laboratory Animal Research (ILAR, 1996). The *Guide* recommends consideration of alternatives (for all animal-based research, not just research that has the potential to cause distress or pain), emphasizes the importance of minimizing distress, and offers examples of procedures that have the potential to cause distress and pain that "cannot be reliably controlled". These include physical restraint, multiple major survival surgery, food or fluid restriction, use of adjuvants, use of death as an end point, use of noxious stimuli, skin or corneal irritancy testing, allowance of excessive tumor burden, intracardiac or orbital-sinus blood sampling, or the use of abnormal environmental conditions (ILAR, 1996). While providing some useful, if disparate examples, the *Guide* does not clarify what is encompassed in its concept of distress, despite the fact that the term is used throughout the text.

A direct result of the 1985 amendments to the AWA and the changes in PHS policy that occurred at around the same time was the establishment of the system of IACUCs. IACUCs have been specifically charged with prioritizing the reduction of pain and distress that may be experienced by animals used in research. IACUCs review protocols submitted by Principal Investigators (those who conduct research, termed 'PIs'), and evaluate the proposed standards of care provided to the animals used as subjects in the study. IACUCs are also required (by the USDA regulators who oversee the conduct of animal research) to ensure that investigators have searched for alternatives if the research is likely to cause animal pain and distress. This must occur even if anesthetics and analgesics are used to prevent any pain and distress, as mandated by USDA Policy #12 (U.S. Department of Agriculture, 2000). Investigators, however, do not have to demonstrate that they have considered or looked for alternatives if the animal research project is placed in the non-painful category. The implicit message is that animal pain and distress is of greater public concern than animal death (usually via euthanasia).

It is important to emphasize that those institutions that use animals not regulated by the USDA and do not receive federal funding are completely unregulated. Some research institutions seek accreditation from the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. The *Guide* serves as the basis for the assessment. Accredited facilities are site-visited and assessed every three years. Though important in encouraging adherence to industry standards, the process is voluntary—facilities are not mandated to seek or maintain AAALAC accreditation.

***The international scene***

As mentioned above, institutions using animals regulated by the Animal Welfare Act in the U.S. are required to submit a report annually to the USDA regarding how many of each species fall into three pain and distress categories that focus on whether anesthetics, analgesics or tranquilizing drugs were used. Any meaningful pain and distress classification system should recognize that there are methods of relieving suffering other than the use of anesthetics and analgesics. For example, antibiotics could be used to reduce any distress and discomfort associated with an infectious disease.

A number of countries require reporting of animal use statistics—these reporting systems are shown in Figures 5. As can be seen, many countries require reporting of levels of pain and distress experienced by the animals. The USDA proposed such a reporting system in the US, which The Humane Society of the United States supports.

If a reporting system that reflects severity of pain and distress the animals experience was adopted, trends in pain and distress could be monitored across institutions and over time and could provide data for comparisons with countries with similar systems. Also, IACUCs could use the new system to gauge the

intensity of the review needed for proposed research, with protocols in the severe category being assigned the most intense review. Following the trends in the severe category would also inevitably lead to a focus on how procedures in that category could be refined in order to be placed in the "moderate" category and, perhaps, ultimately in the "minor" category. This would lead to a true focus on the Three Rs of replacement, reduction, and refinement.

**The Ethical Context**

The use of animals in research is an ethically charged subject. The philosophical arguments are generally broken down into two major camps: deontological and utilitarian (Rowan and Loew, 1995). The deontological argument in general is that nonhuman animals have certain rights that cannot be over-ridden, with some arguing that this means they have a right not to be used for research purposes at all, or at least should receive the same consideration as humans. It is important to note that humans were once used in harmful research without any restrictions, but steps have been taken to increase protections, based largely on ethical arguments. For example, the United States has federal legislation and regulations that protect human research subjects. Internationally, the Declaration of Helsinki (World Medical Association, 2004), developed by the World Medical Association provides principles in regards to the use of human subjects. The principles discuss various issues, including that the human's interest is first priority, that harms should be weighed carefully with the human subject's well-being in mind, and that the protocol "should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present declaration are complied with."

The utilitarian view in general considers harms and benefits of the proposed research using animals, with the ethically preferable result being the greatest good for the greatest number (Rowan and Loew, 1995). This leads to disagreements over degrees of harm and benefit. For example, animal researchers will opine that the use of animals produces great benefit in comparison to the costs. Opponents of animal research, on the other hand, argue that animal research incurs great cost to the animals with little benefit and that most animal research shouldn't be carried out at all (Singer, 1990) .

Rollin (1981) takes a different approach and combines the deontological and utilitarian points of view. He argues that if it is decided to use animals for a research purpose after consideration of potential benefit (utilitarian), then the animal has a right to be protected to the extent possible (deontological). Therefore, if the study causes pain, the animal has a right to be protected and should be given analgesics.

While most of the discussion of the ethical status of animals can be boiled down into the two main categories mentioned, Tannenbaum and Rowan (1985) argue that philosophical considerations regarding the use of animals in research have become more carefully formulated over time and they offer a total of six ethical positions to reflect this:

1. Ethical skepticism and relativism: The root of ethical skepticism is that moral claims cannot be true or false. Consequently, any moral claims against animal research are simply expressed opinions and desires that are not right or wrong and, furthermore, do not serve as a basis for ending animal research. The root of ethical relativism is that right and wrong are subjective and dictated by culture. In terms of animal research, according to this view, because most people support animal research, it is justified.

2. Absolute dominionism: This is the view that there are no moral constraints on the human use of nonhuman animals. Consequently, certain types of research would not have more ethical value, per se, than others and cosmetics testing is just as acceptable as cancer research.

3. Anthropomorphic consequentialism: According to this position, how nonhumans are treated by humans actually impacts the well-being of humans. In regards to this position, animal research that causes severe pain simply out of intellectual curiosity would be unacceptable.

4. Reverence for life: This is based on the notion that all living beings exhibit a will to live and that life should be cherished and only caused harm if necessary. This position is largely based on the work of Albert Schweitzer, who did not clarify this position in terms of the use of animals for research. The term "necessary" however, is subjective.

5. Human beneficence: This is the view that no unnecessary pain or distress should be inflicted upon animals, but that animals do not have an inherent right to live and therefore can be euthanized for "legitimate purposes." Again, the term necessary is subjective.

6. Utilitarianism: See the discussion of utilitarianism above.

7. Deontological: See the discussion of deontological arguments above.

While consideration of these various positions is interesting and should be reflected upon, the public demands that the welfare of the animals currently being used must be properly addressed. As a result, researchers are typically required (by law) to justify animal use (in other words, the potential benefits of the work) and to minimize harm to the animals (in other words, the costs to the animals). Benefits of animal research are largely considered in terms of overall value to human health; public perception of the value of research to human health (the benefit) is key to public support. For example, public support is greater for research related to disease than testing of cosmetics.

Costs of research are considered in terms of costs to the animals, particularly the distress and pain that they experience. The public is not only demanding that researchers minimize harm, but that severe harm to the animals should not be allowed, regardless of the potential for human benefit (The Humane Society of the United States, 2001). As Rollin (2000) points out when examining the issue of research that causes severe animal suffering, such research "is no longer ethically acceptable to today's social ethic for animals" and that failure to move away from these methods will negatively impact scientific credibility and could have legislative consequences.

**Moving Forward:**

*Concepts of distress*

As mentioned, the first section of this book addresses concepts of animal distress. This discussion begins with a chapter by Rollin regarding the philosophical issues surrounding animal pain, distress and consciousness; including ethical considerations and information regarding how scientists and society have increasingly tackled these issues in recent decades. Following this philosophical discussion, Dawkins discusses concepts of distress and suffering and how these can be interpreted operationally. Dawkins frames the concept of distress by asking two questions—are the animals healthy, and do they have what they want? Fraser subsequently addresses whether animal distress can actually be assessed and/or measured. Specific case examples are provided to describe signals, indicators, or indirect measures (such as physiological measures) that an animal is perhaps experiencing a negative affective state. An animal's preference, motivation, and active avoidance, as well as presence of abnormal behavior or absence of normal behavior, are considered as means of assessing affective states.

With the groundwork laid on operational interpretation and whether distress can be assessed, Duncan addresses suffering versus distress and the importance of considering pleasure in addition to distress in order to assess an animal's well-being. Richmond concludes the first section by explaining various international regulatory definitions of distress in animal research and animal production.

*Toward an agreed concept of distress*

In July of 2000, following the launch of The HSUS's Pain and Distress Campaign, the USDA published a proposal (*Federal Register*, Docket # 00-005-1) seeking comment on whether the term "distress" should be defined and, if so, what the elements of the definition should be. The USDA has publicly indicated that, based on the comments submitted in response to the proposal, there is no consensus within the research

community on what the definition of distress should be. However, in an exhaustive analysis of all the comments submitted by members of the scientific community, The HSUS found that, for all practical purposes, there is consensus among this community. Although most researchers indicated that they do not support the adoption of a regulatory definition of distress, 96.6% indicated that *if* the USDA does define distress, then the National Research Council (NRC) definition should be used (see Appendix D of this chapter for details of the analysis and Appendix E for additional definitions that were proposed). The NRC definition is as follows: "an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and shows maladaptive behaviors" (ILAR, 1992). While this definition has its limitations (e.g., it refers only to overt behavior), the USDA overlooked its appeal to the research community and missed an opportunity to solicit comments on ways in which this definition could be improved. The USDA has not yet officially moved forward on this distress-related proposal (as of August 2009).

Given that the USDA and the National Institutes of Health's Office of Laboratory Animal Welfare (OLAW) are the two main animal research oversight agencies in the United States, the task of minimizing, and eventually eliminating, animal distress and pain can be facilitated by the improvement of standards governing animal-based research by these agencies.

*Recognition and Alleviation of Pain and Distress: Problems and Technical Issues*

The systematic reduction of animal pain and distress in the research laboratory is obviously not a trivial task, for several reasons. First, there is much conceptual confusion in the use of such terms as pain, distress and suffering. Second, animal use in the laboratory and classroom is very varied. Third, animals are complex organisms, so animal pain, distress and suffering are not easy to recognize or measure unambiguously across species.

These obstacles are not insurmountable, however. Terminology can be clarified. While the techniques used in biomedical research are certainly numerous, it is certainly not beyond our ability to determine underlying principles of pain and distress in animals which can then be applied to the varied models and methods. And while there is considerable opportunity for legitimate disagreement among scientists over distress and pain issues within and across species, there is also a growing body of scientific understanding of how systems underlying animal distress interact.

Aversive or distressing stimuli can take a diverse variety of forms. Some stimuli are physiological stressors (e.g. injury, surgery, disease, starvation and dehydration), some are psychological stressors (e.g. situations that induce fear, boredom, anxiety), some are environmental stressors (e.g. restraint, excessive noise, the presence of people or other species and chemicals) and some are a mixture of one or more of these factors. Assessing the severity of resulting adverse states is a complex task. This, however, is a task that must be addressed.

In biomedical research, animals may experience pain, discomfort, anxiety, fear, and a variety of other adverse states, in addition to functional deficits caused by experimental procedures. In most experimental protocols, an animal's pain may be treated with anesthesia and analgesics. These measures may relieve or even eliminate the experience of pain. To date, however, there are no similarly well known methods to alleviate non-pain induced distress an animal is subject to before, during, or after experimental procedures. In some experimental protocols, anesthesia or analgesics are thought likely to interfere with the results and are therefore not used, leaving the animal with persistent and unrelieved pain, which can lead to chronic and unrelenting distress, which may *further* impact scientific results (Carstens & Moberg 2000). If distress not caused by pain isn't relieved, it can also impact results. There is thus both an animal welfare and a scientific need to understand animal pain and distress as well as the relationship between the two

Pain researchers have paid attention to the use of limited levels of painful stimuli in animals. For example, the International Association for the Study of Pain (IASP) has set up guidelines in which researchers are urged to design only projects in which animals are given the opportunity to terminate any painful stimulus and thus control the level of pain they experience (IASP, 1979). Similarly, one can

develop systems that allow animals to "volunteer" for research procedures by offering them a highly desired food or drink (Laule, Bloomsmith & Schapiro 2003). Such animals are willing to accept some painful stimuli in order to gain the reward. However, at the pain tolerance threshold, they voluntarily choose not to participate any further (Ron Dubner, personal communication, 1991). In all of the above cases, the research protocol allows the animal to control the situation by terminating the painful stimulus. There are some studies (e.g. of chronic pain) where such refinements are not possible but even here pain researchers have tried to ensure that the animals do not endure a significant level of pain, such as by exposing the animals to the minimal pain necessary in order to conduct the study or using analgesics when possible (Casey & Dubner, 1989). Pilot studies are also recommended in order to determine how to limit the duration of pain while still meeting scientific objectives, for example (Casey & Dubner 1989). By contrast, researchers who study anxiety in animals, which is arguably just as, if not more distressing to animals, have only just begun to consider developing similar guidelines and approaches. Some may not have paid attention to animal anxiety because they do not believe that animals can be anxious although they can experience the more “primitive” emotion of fear (e.g. Cassano, 1983). Yet anxiety is recognized by respected scientists as likely to be a key aspect of distress (e.g. National Research Council, 2003)

Despite the various difficulties regarding animal pain and distress, the experts who have contributed to this report provide readers with extensive information currently available on causation, recognition, measurement, and alleviation of laboratory animal distress in the second half of this book. This discussion begins with a chapter by Carbone regarding various causes of animal distress in the laboratory. These causes can be related to husbandry, the actual study being conducted, disease, deprivation of something the animal needs, phenotype and genotype, and so on. A discussion of how to prevent and/or address these sources of distress is included. Morton provides an initial discussion surrounding concepts of distress and then focuses on specific ways to measure and assess distress, including the use of score sheets. Hampshire further explores assessment of distress and provides valuable information on the role of clinical veterinary medicine in resolving animal distress, such as by conducting physical exams and tracking and interpreting results in order to appropriately treat the animal to relieve distress and pain. The authors of chapter ten provide practical information on specific procedures or areas of research, such as infectious disease, psychological disorders, carbon dioxide euthanasia and polyclonal antibody production, and discuss practical means of recognizing and alleviating animal pain and/or distress in these models. Finally, Conlee, Stephens and Rowan provide conclusions and recommendations on how to minimize animal pain, distress and suffering in the context of research and testing.

*Specific models and areas of research causing animal pain and distress*

Pain and distress caused by specific research models and techniques raises serious concerns for those in the scientific community as well as in the animal welfare community. Good prospective estimates of how much animal pain and/or animal distress is caused by particular techniques or methods (with empirical evidence to support the estimates) are not yet available in many cases. For this very reason, gathering data during these studies to discriminate amongst research models and specific techniques is essential. Additionally, the extent and type of pain and distress may be specific to a particular research model, while other factors like animal species or gender may affect the extent of suffering caused within that particular animal model.

Much attention has been focused on the use of animals in the testing of personal care and household products although such use probably accounts for much less than one percent of the national demand for laboratory animals. Within industry, the vast majority of animal use is involved in the discovery, development and testing of new medicines and therapeutics. For example, an analysis reveals that in 1998, 92,405 animals were reported as experiencing unrelieved pain and distress in testing procedures, specifically, in the United States, and vaccine potency testing alone accounted for approximately half of these procedures Disturbingly, Category E descriptions (the most serious category of distress reported) typically are too brief and generalized to permit a more detailed analysis of the procedures involved (Stephens, et al., 1998). These findings are similar to those of Stephens, et al. (1998).As mentioned, the authors of chapter thirteen provide practical information on specific areas of research, including toxicity

testing, psychological models, carbon dioxide euthanasia and polyclonal antibody production as well as the influence of surgical skills on resulting pain and distress.

## Conclusion

Pain and distress in animal research is a topic of importance to the public, animal protectionists and scientists alike. Over time, attitudes toward animal research have shifted; animal research is becoming less accepted, and some practices considered to be acceptable in the past are no longer considered so (Mukerjee, 1997). However, it is evident that animal pain and distress are not being addressed as they should, either by regulatory agencies or research institutions. The public's extensive interest in the humane treatment of animals in laboratories prioritizes the need for greater attention to refinement techniques, best practices, reporting systems and other regulations pertaining to animal pain and distress, as well as focus and sustained effort to eliminate animal pain and distress. Work in these areas would serve to significantly improve the situation for animals used in the laboratory.

The responsibility to minimize research animal distress and pain lies with the researchers, other laboratory personnel, Institutional Animal Care and Use Committees, and the agencies that oversee research animal welfare. As long as animals continue to be used for biomedical research in an attempt to improve human health, these entities should fulfill their ethical and scientific obligation to relieve the animals' pain and distress (Robertson, 2002). Consequently animal welfare should be made an urgent priority. This book examines many of the means by which scientists and others can move toward achieving these goals.

In the past few years, there has been an increase in attention to pain and distress issues within science and academia, as evident by increased use of analgesics and increased sessions devoted to pain and distress at scientific conferences, for example, as discussed earlier in this chapter. The result is genuine progress in the form of experimental data addressing animal pain, distress, and well-being and an increase in the debate about the conceptual issues. These activities will lead to improvements for both animals and the humans that rely on them, but they must be pursued vigorously, with a positive and constructive debate on animal distress within both the public and scientific arenas. This book seeks to enhance this pursuit through discussion of conceptual and practical issues regarding animal pain and distress in the laboratory. In the end, better animal welfare will lead to better science; unless unwanted factors such the pain and distress are eliminated, they will always confound scientific data and ultimately translate into poorer human welfare as well.

**References**

Aldhous, P., Coghlan, A., and Copley, J. (1999) Let the People Speak. *New Scientist*, 22 May, pp.26-31.

American College of Veterinary Anesthesiologists (2006) American College of Veterinary Anesthesiologist's position paper on the treatment of pain in animals. Retrieved August 4, 2009 from http://www.acva.org/professional/Position/pain.htm

Anand, K.J. and Hickey P.R. (1987) Pain and its effects in the human neonate and fetus. *New England Journal of Medicine*, 317(21): 1321-1329.

Balcombe, J.P., Barnard, N.P., and Sandusky, C. (2004) Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science, 43*, 42-51).

British Union for the Abolition of Vivisection (2003, Winter) Report (pp. 20-21). London, United Kingdom: BUAV.

Byrne, R. (1999) Primate cognition: evidence for the ethical treatment of primates*. In Attitudes to Animals: Views in Animal Welfare*, ed. Francine L. Dolins, Cambridge University Press, Cambridge, England, pp. 114-125.

Canadian Council on Animal Care (nd) Module 10: analgesia. Retrieved August 4, 2009 from http://www.ccac.ca/en/CCAC_Programs/ETCC/Modcue10/toc.html

Carstens, E. and Moberg, G. (2000) Recognizing pain and distress in laboratory animals. *Institute for Laboratory Animal Research* 41(2): 62-71.

Casey, K.L. and Dubner, R. (1989) Animal models of chronic pain: scientific and ethical issues. *Pain*, 38:249-252.

Cassano, G.B. (1983) What is pathological anxiety and what is not? In The *Benzodiazepines: from Molecular Biology to Clinical Practice*, ed. E.Costa, pp 287-293. New York, Raven Press.

Cassell, E.J. (1982) The nature of suffering and the goals of medicine. *New England Journal of Medicin,e* 306:639-645.

Cassell, E.J. (1989) What is suffering? *In Science and Animals: Addressing Contemporary Issues*, eds. H. N. Guttman, J.A. Mench, and R. C. Simmonds, pp. 13-16. Bethesda, MD; Scientists Center for Animal Welfare.

Damasio, A. R. (1994) *Descartes' Error: Emotion, Reason and the Human Brain*. New York: G. P. Putnam's Sons, 344 pages.

Hampshire, V., Davis, J. and McNickle, C. (2000) Red-carpet rodent care: making the most of dollars and sense in the animal facility. *Lab Animal*, 29, 40-46.

Herzog, H., Rowan, A., and Kossow, D. (2001) Social attitudes and animals. *State of the Animals 2001*. Washington, DC: Humane Society Press.

Hockly, E., Cordery, P.M., Woodman, B., Mahal, A., van Dellen, A., Blakemore, C., Lewis, C., Hannan, A.J., Bates, G.P. (2002) Environmental Enrichment Slows Disease Progression in R6/2 Huntington's Disease Mice. *Annals of Neurology, 51*, 235-242.

IASP (1979). Report of International Association for the Study of Pain; Subcommittee on taxonomy. *Pain*, 6:249-252.

Institute for Laboratory Animal Research (ILAR) (1992). *Recognition and Alleviation of Pain and distress in Laboratory Animals*. Washington, DC; National Academy Press

Institute for Laboratory Animal Research (1996) *Guide to the Care and Use of Laboratory Animals*. Washington, D.C.: National Academy Press.

Institute for Laboratory Animal Research (2008) *Recognition and Alleviation of Distress in Laboratory Animals*. Washington DC: National Academy Press.

Kitchen, H., Aronson, A.L., Bittle, J.L., McPherson, C.W., Morton, D.B., Pakes, S.P., Rollin, B.E., Rowan, A.N., Sechzer, J.A., Vanderlip, J.E., Will, J.A., Clark, A.S. and Gloyd, J.S. (1987). Panel report on the Colloquium on recognition and alleviation of animal pain and distress. *Journal of the American Veterinary Medical Association,* 191:1186-1191.

Laule, G.E., Bloomsmith, M.A. and Schapiro, S.J. (2003) The use of positive reinforcement training techniques to enhance the care, management, and welfare of primates in the laboratory. *Journal of Applied Animal Welfare Science*, *6*,: 163-174.

Marquie, L., Raufaste, F., Lauque, D., Marine, C., Ecoiffier, M., and Sorum, P. (2003) Pain rating by patients and physicians; evidence of systemic pain miscalibration. *Pain*, 102 (3):289-296.

Mukerjee, M. (1997) Trends in animal research. *Scientific American*, 276, 86-93.

National Research Council (2003) *Guidelines for the care and use of mammals in neuroscience and behavioral research*. National Academy Press: Washington, D.C.

National Science Board (2002) Public attitudes toward the use of animals in scientific research. Retrieved August 4, 2009 from www.nsf.gov/statistics/seind02/pdf/c07.pdf

OECD (2000) *Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation*. Organisation for Economic Co-operation and Development, Paris, France.

Page, G.G. (2002) Immune-suppressive effects of pain. In Halina Machelska and Christoph Stein (Eds) *Immune Mechanisms of Pain and Analgesia*. Springer: New York.

Paul-Murphy, J., Ludders, J.W., Robertson, S.A., Gaynor, J.S., Hellyer, P.W., Wong, P.L. (2004) The need for a cross-species approach to the study of pain in animals. *Journal of the American Veterinary Medical Association*, 224(5): 692-697.

Phillips, M.T. (1994) Savages, drunks and lab animals: the researcher's perception of pain. *Society & Animals*, 1: 61-81.

Plous, S. (1996) Attitudes toward the use of animals in psychological research and education: results from a national survey of psychologists. *American Psychologist*, 51: 1167-1180.

Richardson and Flecknell (2005) Anaesthesia and Post-operative Analgesia Following Experimental Surgery in Laboratory Rodents: Are We Making Progress? *Alternatives to Laboratory Animals*, 33: 119-127.

Robertson, S.A. (2002) Pain management in laboratory animals--are we meeting the challenge? *Journal of the American Veterinary Medical Association*, 221, 205-208.

Rollin, B.E. (1981) *Animal Rights and Human Morality*. Buffalo, NY: Prometheus Books.

Rollin, B.E. (2000) An ethicist's commentary on research protocols requiring significant animal suffering. *Veterinary medical ethics*, 41: 748.

Rowan, A.N. and Loew, F.M. (1995) *The Animal Research Controversy*. Grafton, Massachusetts: Tufts University School of Veterinary Medicine, Center for Animals & Public Policy.

Rowan, A.N., Stephens, M.L., Dolins, F., Gleason, A. and Donley, L. (1998) Animal welfare perspectives on pain and distress management in research and testing. In *Proceedings for Pain Management and Humane Endpoints*. Retrieved August 4, 2009 from http://altweb.jhsph.edu/meetings/pain/rowan.htm

Seligman, M.E.P. (1975). *Helplessness: on Depression, Development, and Death*. W.H. Freeman, San Francisco.

Singer, P. (1990) *Animal Liberation*. (2nd ed.) New York, NY: Random House, Inc.

Stephens, M.L., Mendoza, P., Weaver, A. and Hamilton, T. (1998). Unrelieved Pain and distress in Animals: An Analysis of USDA Data on Experimental Procedures. *Journal of Applied Animal Welfare Science*, 1(1):15-26.

Stephens, M.L. and Conlee, K.M. (2004) The HSUS's Pain and Distress Initiative: overview and update. *Alternatives to Laboratory Animals*, 32: 423-427.

Tannenbaum, J. and Rowan, A.N. (1985) Rethinking the morality of animal research. *Hastings Center Report*, 15(5): 32-43.

The Humane Society of the United States (2001) Opinion poll on pain and distress in research. Retrieved August 4, 2009 from www.hsus.org/animals_in_research/pain_distress/opinion_poll_on_pain_and_distress_in_research.html

The Humane Society of the United States (2003, December) United Kingdom Joint Working Group should be model for the United States. Pain & Distress Report, 4(1): 3. Retrieved August 4, 2009 from http://www.hsus.org/web-files/PDF/ARI/pd_reports/P_and_D_Report_Dec03.pdf

The Humane Society of the United States (2004, June) Evidence of increased attention to pain and distress issues. Pain & Distress Report, 4(2): 1. Retrieved August 4, 2009 from www.hsus.org/web-files/PDF/ARI/pd_reports/P_and_D_Report_Jun04.pdf

U.S. Department of Agriculture (1997) Policy #11: Painful/Distressful procedures. Retrieved on August 4, 2009 from www.aphis.usda.gov/animal_welfare/downloads/policy/policy11.pdf

U.S Department of Agriculture (2000) Policy 12: consideration of alternatives to painful/distressful procedures. Retrieved August 4, 2009 from www.aphis.usda.gov/animal_welfare/downloads/policy/policy12.pdf

World Medical Association (2004) World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. Retrieved August 4, 2009 from www.wma.net/en/30publications/10policies/b3/index.htm

Figure 1.