2009

Measurement and Mitigation of Laboratory Animal Distress Sources of Distress in the Animal Laboratory

Larry Carbone

Measurement and Mitigation of Laboratory Animal Distress Sources of Distress in the Animal Laboratory

Larry Carbone

Some definitions

Distress in laboratory animals is a serious welfare and ethical concern. It can also be a serious consideration in interpretation of research data, as the stressed (or distressed) animal differs in multiple ways from the unstressed subject.

Pain and distress differ, but overlap. For the purposes of this discussion, we will consider pain to involve nociceptive input of stimuli that are potentially tissue damaging, and that further include an unpleasant emotional component (Merskey and Bogduk 1994). Pain need not necessarily induce distress, as when an animal or human willingly undergoes some painful situation in order to achieve a desired reward. In that case, while the pain may be unpleasant, it is not so severe as to be intolerable. Likewise, there are many potential causes of distress that do not involve physical pain.

Distress also differs from stress. Animals experience a variety of stressors, some of which may actually be pleasurable to the animals (hence the neologism “eustress”). The word *distress* applies when an experience is significantly unpleasant to the animal. Moberg has argued that multiple small “subclinical” stresses may combine to push the animal to the level of distress – for example, a change in diet here, exposure to cold temperatures there, a minor surgery and anesthesia. Multiple small challenges to which the animal could normally respond may act synergistically to a point where “the stress response shifts sufficient resources to impair other biological functions” (Moberg 2000). The animal’s response is key here – distress should never be thought simplistically as simply the sum of many stressors. Indeed, single events may result in animals distress.

I avoid the qualification that the terms “distress” or “dystress” only apply when the situation is greater than the animal’s ability to cope (Morton 1998). Despite efforts to clarify what coping means in animal welfare (Broom 1998), it seems to me that the term distress applies long before a situation arrives at being beyond the animal’s ability to cope (whatever that may actually mean).

Moral and legal responsibilities

In the United States, the Animal Welfare Act requires research investigators to consider alternatives and to consult with veterinarians regarding any procedures that may cause species of animals that are covered by the act more than momentary or slight pain or distress (United States Congress 1985). The Public Health Service Policy on Humane Care and Use of Laboratory Animals lays a similar obligation, though for all vertebrate species (Office of Protection from Research Risks 1986).

Notice that there is a threshold implied in the current regulations. The Animal Welfare Act’s threshold is clear in the case of pain: “pain in excess of that caused by injections or other minor procedures (Animal and Plant Health Inspection Service 1989).” This threshold’s significance arises from the associated obligation for alternatives searches, for veterinary consultation, and for annual reporting that it entails. No comparable threshold for distress has been developed.

I believe our moral obligation to laboratory animals goes far beyond merely preventing severe pain or distress that exceeds some regulatory threshold. We owe these animals the very best we can provide for them, a moral commitment that is intimated in the Animal Welfare Act's provisions for psychological well-being and even for dog exercise (United States Congress 1985). This is the goal of enrichment programs that aim for animals' ability to conduct a wide range of species-typical behaviors (Mench 1994; Burghardt 1996). This is also the impulse that underlies efforts to place laboratory animals in adoptive homes (Carbone, Guanzini et al. 2003). Indeed, the ILAR *Guide for the Care and Use of Laboratory Animals* implies a regulatory mandate to go beyond the prevention of distress, in its recommendation that "animals should be housed with a goal of maximizing species-specific behaviors" (Institute of Laboratory Animal Resources 1996).

While I believe we have a moral and legal obligation to go far beyond preventing distress, to aim for the happiest possible laboratory animals, that does not mean that animals in relatively impoverished environments are necessarily suffering or in significant distress. That said, if Moberg was correct that many small stresses can lead to significant distress, then we really must try to prevent most of these small stresses so that animals are not overwhelmed.

Categorizing distress in the animal laboratory

There are many ways to categorize the sources of distress in the laboratory. The categories can organize our thinking and provide a checklist of concerns to cover; they are not all mutually exclusive. Building on the work of Russell and Burch (Russell and Burch 1959), Table 1 illustrates some of these categories, but does not imply that everything in an animal's life that could fit into one of these categories is going to be distressful.

Pain, disease, and distress

Pain and disease can both cause distress and suffering in laboratory animals, so it (almost) goes without saying that everything we can do to prevent, or at the least, to treat, pain and disease will prevent or reduce distress. Of course, it is not quite so simple.

First, it must be recognized that anesthesia itself (especially the induction and recovery periods) is a potential stressor in animals' lives (Hedenqvist, Roughan et al. 2001). Likewise, analgesic drugs can have significant side-effects (SoRelle 2004; Thompson, Kristal et al. 2004). Neither of these concerns necessarily argues against the use of anesthetics and analgesics in the face of painful procedures. Rather, drug effects and the patient's status must be considered, and often, concern for drug side-effects suggests the use of a greater number of drugs, to reduce the doses of single drugs and thereby maximize pain management while minimizing risk. Thus, for a typical major primate surgery, the prescribed balanced anesthesia and multimodal analgesia for a single patient may include ketamine, midazolam, isoflurane, nitrous oxide, lidocaine, bupivicaine, meloxicam and buprenorphine.

Anesthetics and analgesics are often necessary to reduce pain and suffering in animals, but it is far better to avoid potentially painful manipulations than to treat the pain with drugs. When these drugs must be used, they must be used correctly in ways that minimize distress.

Pain-related distress cannot be entirely eliminated in research protocols. But it should be predictable, with good protocol review, and so plans to manage it should be in place before it even begins (Wolfle 2000). Monitoring parameters, as well as the timing and frequency of

monitoring, should be reviewed by the Institutional Animal Care and Use Committee. Animal protocols are not set in stone; if they are not proceeding as predicted, they must be reassessed and modified as needed. Annual protocol renewal is a good time to ask investigators to account for unanticipated adverse events, though significant adverse events should be dealt with as they present.

In addition to pain management related to surgical procedures, I want to emphasize that pain management should be a component in clinical management of many illnesses as well. Cage injuries as well as many spontaneous infections and diseases must be diagnosed and the primary condition treated, of course, but while the animal waits for a twisted ankle to heal, for antibiotics to successfully reduce infection, for a needed dentistry, there is potential for pain and associated distress, and an obligation to treat that pain. Not every infection, tumor, or other illness causes pain, but if there is any significant degree of inflammation as part of the disease, then there is significant potential for the use of nonsteroidal or other analgesics to minimize pain and distress. Recent advances in long-acting, potent, palatable non-steroidal anti-inflammatory drugs and opioids greatly enhance our ability to follow this general principle: when in doubt, we should err on the side of over-treating rather than under-treating pain.

Phenotypes and genotypes

Russell and Burch distinguished what they referred to as direct as opposed to contingent “inhumanity” (Russell and Burch 1959). Direct inhumanity was the suffering induced by the experiment and required by the experiment (as when explicitly studying pain or distress). Contingent inhumanity is the suffering that occurs as an undesired side effect of the research manipulations, or from husbandry and environmental challenges.

Two and more decades ago, surgical pain was the over-riding non-husbandry concern in laboratory animal welfare guidelines (Hume 1957; Animal Care Panel 1963). Hygiene and infection control were the husbandry issues. All of that has changed with the proliferation of mice (and other animals) which harbor a range of inborn genetic diseases and disorders.

It did not take transgenic technology for this to occur. Selective inbreeding of mice with spontaneous mutations set the stage (Quimby 2002). Before gene transfer was developed, there were strains of mice and rats with such inborn diseases as autoimmune hemolytic anemia (NZB mice), high tumor incidence (Fischer 344 rats) and severe immune deficiencies (nude mice and rats). The hunt for spontaneous mutations was never restricted to laboratories or to rodents, but has ranged over farm and companion animal practice. Household dogs with spontaneous mutations have founded lines of dogs with Ehlers-Danlos syndrome (collagen deficiency), X-linked muscular dystrophy, Hemophilia A and B, and a host of congenital heart defects (Committee on Dogs 1994).

Regardless of the source of the underlying genes, many animal phenotypes may result in inherent and significant distress, disease, or pain to the animals. The phenotype determines both the condition and the needed management. Immunodeficient rodents can be kept in good health as long as they are shielded from opportunistic pathogens; this may be achieved by using sterilized microisolator cages, with sterilized food, water, bedding and cage enrichments. Hypoglycemic strains of mice may be maintained in relatively good health if fed 5% glucose in their drinking water (Mandriota, Jussila et al. 2001). Hemophiliac dogs require special housing and handling to minimize injuries, careful examination for evidence of internal bleeding, and maintenance of emergency transfusion products (Dodds 1992; Committee on Dogs 1994).

Though the source of the gene does not determine the phenotype, or the attendant welfare concerns, transgenic technology is nevertheless of special concern for several reasons:

- There is a rapid proliferation of illness-inducing genotypes now that we no longer wait for spontaneous mutations. This requires similar speed in learning to manage new phenotypes humanely.
- After decades of increasingly centralized breeding of laboratory rodents in a number of specialized institutions with very high quality-control standards, development and maintenance of new strains is once again decentralized. Fifteen years ago, conventional rodent stocks, maintained on research campuses or in individual laboratories along with their share of conventional pathogens, seemed on their way to obsolescence. Now, the proliferation of 'boutique' strains of animals maintained far from the quality-controlled facilities of Jackson Laboratories or its commercial equivalents has spawned a brisk inter-institutional trade in animals and with this, a great risk of spreading pathogens from one facility to another. Even with infections that are usually non-pathogenic, management of an outbreak can require wholesale depopulation. Furthermore, some infections may prove to be more pathogenic in some modified strains, or harder to eradicate.
- Anecdotal evidence suggests that transgenic technology has led to an overall increase in the numbers of mice being maintained (Carbone 2004). Statistics are unavailable to back up this claim, partly because in the USA, laboratory-bred mice are "exempt" from the USDA's reporting requirements. In 1959, Russell and Burch worried that there is some theoretical threshold population beyond which there is a "problem of scale" (p. 65-6) (Russell and Burch 1959): no matter how conscientious the staff, the ability to truly attend to animal welfare is strained with increasing population size. If any development is to test their prediction, it will be the current rise in transgenic technology.
- Finally, the technology of producing even the most benign of transgenic strains contains the potential for pain and distress. Current technology entails implantation of embryos, a major survival surgery requiring general anesthesia and care during post-operative recovery. Tissues must be sampled from young mice for genetic analysis, and individual young mice must be identified; both of these procedures have traditionally utilized invasive procedures, such as ear-notching, tail amputation, and digit amputation. Improvements in technology will see the refinement of tissue sampling toward less invasive techniques (such as saliva, fecal, or hair sampling). Non-invasive (if temporary) marking methods may suffice to separate the gene-carriers from the non-gene-carriers. A working group has addressed several of these and related welfare concerns in transgenic technology and published their recommendations (BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement 2003).
- On the bright side, developments in the modulation of gene function mean that scientists may implant disease-causing genes, or knock out disease-preventive genes in increasingly controllable ways. An inducible tumor-causing gene may be inserted, for example, but not turned on until the researcher is actually ready to study the condition, or perhaps never turned on at all in the breeding animals. As we increase our power to create new disease models in rodents, at least we might include some degree of selectivity of when the disease will present.

Social interactions of animals

Laboratory animals inhabit a social world that includes conspecifics, whether in their home cage or just within the room, as well as humans. The 1996 *Guide for the Care and Use of Laboratory Animals* says that "whenever appropriate, social animals should be housed in pairs or groups,

rather than individually (p. 26) (Institute of Laboratory Animal Resources 1996). This precept must be applied judiciously. In natural settings, few animals live in complete isolation from their kind, but neither do they live in forced intimacy. Many individual monkeys, for example, may live at the periphery of the troop, neither totally off on their own, nor right in the face of an alpha animal. The social arrangement that works best in the confines of the laboratory will depend on the animal species, on the individual animal, and on the available caging.

Many animals, even those that live solitarily in the wild, will seek out each other's company in captivity. I've seen woodchucks, for example, pile into one nest box together, leaving other boxes completely empty; adult woodchucks are not so gregarious in the wild. A demonstrated preference for sociality, however, does not necessarily mean that the animals would be significantly emotionally distressed by living alone. Nor will all individuals have the same preferences. In one study, whether female rabbits chose to enter a group or a solitary pen depended on their previously-demonstrated rank. Lower-ranking rabbits were more likely to enter a solitary pen (Held, Turner et al. 1995). However, in most assessments of female domestic rabbits' sociality, there is a demonstrable preference for social housing (Gunn and Morton 1993; Morton and al. 1993; Love 1994; Raje and Stewart 1997; Turner, Held et al. 1997; Kalagassy, Carbone et al. 1999); the key may be in how that housing is set up, whether the animals can get needed distance from each other, whether there are visual barriers and areas of escape from dominant animals available, and the individuals' familial, developmental and experiential history.

Inter-animal aggression must be minimized, either by housing animals singly, by carefully matching up individuals and observing their compatibility, or by adding sufficient complexity to the housing to allow displacement activities and a chance for subordinate individuals to hide from dominants. Adult dogs and primates will generally only be formed into social groups if there is sufficient staff available for careful assessment of compatibility. Other species rarely receive this standard of care, possibly because the results of aggression are less obvious. Whereas monkeys and dogs may inflict life-threatening wounds on each other, cats, rats, female rabbits and others are far less likely to do this. Among household cats, elimination disorders (urine spraying, failure to use litter box) may signal social stress (Overall 1997); that this does not typically escalate to bloodshed, or that laboratory staff may be less bothered by cats' toilet habits than homeowners, does not mean that cats are immune from significant distress in some social situations created in the laboratory. Detecting signs of distress in this situation can require close observation, but cases of notable distress may be remedied by altering the social mix of a cage group, or altering cage structure.

Animals often can adapt well to a particular social arrangement, but may then fare poorly when this changes. Change could come from within the group for developmental reasons, for example as group members reach puberty, come into estrus, or challenge a formerly stable hierarchy. Change can also be imposed, as when animals are removed from a group cage for study, euthanasia, etc. Where social changes can be predicted in advance, management of their occurrence should be planned. An experiment in which cohorts of rats' social groupings would be constantly shifting as animals reach endpoint and are euthanized may best be done with singly caged animals, or with creative plans in place. One such plan could include keeping in every cage an untreated 'nurse rat', who is expected to remain healthy and provide social support to the animals receiving the experimental treatment. Similarly, forcing social grouping onto some animals (monkeys for example) may lead to a period of stress that would outlast the animals' tenure at the facility; the distress of forming new social groups may only be justified for animals that will be maintained for several months.

Despite the stress and dangers of social grouping, I believe the *Guide* is correct to name social housing as a general goal for animals of most species (Institute of Laboratory Animal Resources 1996). Those animals who must be caged singly for most of the day might still be candidates for supervised play or social time with conspecifics, with members of compatible other animal species (even dogs and cats can be companionable with each other, even in the laboratory setting, as can some species of primates and various livestock species), or with humans. Grooming partitions and other devices may allow some social interaction with less risk than fully sharing a cage.

Acclimation to surroundings and procedures

The best of environments take some time to get used to. Researchers must expect that animals may undergo some temporary stress in their first few days in a new environment, and that this may escalate to distress if they are handled improperly, or subjected to experimental procedures prematurely, before fully habituated (Ruiven, Meijer et al. 1996). An acclimation period prior to major survival procedures accomplishes many goals. It allows staff to screen for signs of ill health, and to learn an individual animal's normal pre-procedural behavior. During this time, animals can be trained to accept treat foods that may become vehicles for oral medications post-procedurally, or whose acceptance or rejection is to be used as an indicator of pain or distress.

Beyond adapting to their surroundings, animals can also acclimate to handling and to experimental techniques. Depending on the species and the age of the animal, gentle handling can acclimate animals to human contact and restraint, and reduce associated fear and distress. But again, caution should be exercised: in some species, and in individuals past a certain window of opportunity for socialization, contact with humans can elicit signs of stress (McMillan 1999). Many animals can, however, learn to cooperate with experimental manipulations, rather than requiring brute force. For example, monkeys can learn to present an arm for injections, yielding both lower levels of distress and reducing stress-related confounds on data (Reinhardt 1991).

There may be pharmacologic means of augmenting the process of acclimation to research procedures, though I have not seen much published to date in support of this. For example, while some sedatives and anesthetics are themselves inherently stressful, benzodiazepines are used clinically in human anesthesiology for their amnesiac effect. Nitrous oxide and ketamine can play similar roles (Kennedy and Luhmann 2001; Ivani, Vercellino et al. 2003). If the choice of sedative for animal procedures routinely included a benzodiazepine or other appropriate medication, the associated amnesic effect might well block unpleasant memories associated with handling, restraint and surgery.

Primary and secondary enclosures

The quality of the primary enclosure (pen, cage, run, etc.), as well as the room or building in which it sits, have long been a focus for improved animal welfare, in the *Guide*, in the Animal Welfare Act, in UFAW's publications, in the Animal Welfare Institute's publications, and elsewhere (Animal Welfare Institute 1953; Committee on Laboratory Animal Housing 1976; Poole 1999; Reinhardt and Reinhardt 2002). Different documents highlight different concerns. Most of these focus on the quality of animals as models, with the philosophy that good animal care and good science go hand in hand.

The data supporting different recommendations varies in quality and extent. As a general rule, the simpler physical environmental parameters and their impact on animal health and physiology currently are better characterized than questions of animals' behavioral preferences and their impact on animal welfare /distress. Systems are well-defined, for instance, to prevent rodents' contact with adventitious infectious agents, just as the clinical and sub-clinical effects of those infections are well-described (at least, in non-transgenic rodent strains)(Committee on Infectious Diseases of Mice and Rats 1991).

Likewise, thermal neutral zones have been identified for many species and it is clear that maintaining animals at temperatures outside of these zones is a stressor (Gordon 1990). What can be done about such a potential stressor? The obvious answer is to maintain animals in temperatures that are within their thermoneutral zone, but when this is not possible animals can be given as much control within their environments as necessary to meet their own needs. For cold stress, this could mean having a range of bedding and nesting materials, having a nook in which to escape drafts, or having companion animals to cuddle up with. Not all stress-reducers are equal however. So, while the thermoneutral zone for an animal can be modified through physiological adaptation (changes in coat thickness, changes in thyroid activity, etc.), that adaptation is unlikely to occur (in most domestic species) unless they first experience sufficient cold stress to trigger it.

The size and configuration of the enclosure can affect animal stress and well-being. In the Animal Welfare Act and the *Guide*, so-called performance standards for cages co-exist with engineering standards, but not always harmoniously. Thus the USDA's animal welfare regulations stipulate a performance standard that dogs and cats be able to "walk in a normal manner" (Animal and Plant Health Inspection Service 1991), but enforce an engineering standard that requires a dog cage only six inches longer, on average, than the dog therein. Even a Chihuahua needs more than six inches to walk in a normal manner. The 1996 *Guide* calls for cage heights that allow "typical postures" (Institute of Laboratory Animal Resources 1996), but no adult New Zealand White rabbit could achieve an upright exploratory posture in a 14-inch cage (Carbone 2004). In twenty years of laboratory animal care, I have yet to encounter a USDA inspector or a site visitor with the Association for the Assessment and Accreditation of Laboratory Animal Care who questioned animal cages that met the 'engineering standards' of the *Guide* or the Animal Welfare Act, but failed to meet the 'performance standards'.

As a general rule, I would argue that animal cages should have both sufficient size and complexity to allow the animals to indulge a range of behaviors that will change over the course of the day. This is not possible in a bare box. Rodents may want to spend part of their day exploring, part of their day nestled in a safe confined space. Dogs may want to run for some part of their day, but will want a comfortable bed for most of it. As we limit animals' options in smaller and plainer cages, it is initially a matter of conjecture, but then a subject for empirical study, to determine which restrictions really matter to the animals. Current standard rat housing, for example, sacrifices exploratory options in favor of safe nestling spaces; lowering cage heights below the current 7 inches might shift that balance even further (Martin, Crook et al. 1994). If small cages can meet only one preference or the other, rats may most care about their security needs; some other species of animals might prefer greater space for exploration. In another example, Crouse and colleagues have demonstrated the obvious, that cats will choose soft beds when they're available, along with the less obvious, that they will spend more of their daily time budget sleeping when a soft bed is available (Crouse, Atwill et al. 1995). What are the welfare costs when this preference for a full day's sleep in a comfy bed is not met? At what point does low-grade sleep deprivation escalate to true distress?

Humane Endpoints

Defining humane endpoints may be the most important refinement of research procedures (Hendriksen and Morton 1999). A humane endpoint may come when an animal is euthanized, or when he or she is otherwise removed from study (via treatment of diseases, removal of instrumentation, vacation from food or water restriction, etc.). Like protocol-induced pain, all forms of protocol-induced illness, disease or distress should be realistically predicted in the protocol to the extent possible, and plans laid for their management. Endpoints must be defined for each study, perhaps through a pilot study. Pilot studies may need to cast a very wide net to identify as many possible sources and types of distress – monitoring body temperature, activity patterns, food consumption, body weight, body condition, tumor progression, serum biochemical markers, blood cell counts, and more – so that those parameters most relevant to the particular study can be determined. Toth has argued the value of identifying moribundity in animals: even though animals have likely already undergone significant stress along the way to moribundity and may look too debilitated to even notice their current state, we may yet spare the animals several hours of terminal pain or distress if we can identify their moribund state and end it with euthanasia (Toth 1997). The costs of aggressively monitoring humane endpoints lie in staffing: most animals in the laboratory animal setting are unattended for a good 12 hours or more though the night, making it difficult to catch a rapidly progressing condition in time to limit significant distress through timely euthanasia.

Euthanasia

It is the rare laboratory animal that is not euthanized in the laboratory, either as a required part of an experiment, or as an individual without further use. No matter the pain, distress, or total lack thereof leading up to this point, animal euthanasia can be calm and pain-free, or painful and distressing. Choice of euthanasia method should be spelled out in advance in the protocol, but may need adjustment for an individual animal's circumstances.

As it has evolved, the American Veterinary Medical Association's panel recommendations on euthanasia have improved their recognition of distress beyond just that induced by pain (Beaver, Reed et al. 2001). The AVMA Panel is but one of several efforts to review the best available information of the relative pain and distress associated with different techniques for killing animals (Close, Banister et al. 1996; Close, Banister et al. 1997; Conlee, Stephens et al. 2005).

In the thirty years since the first AVMA Panel recommendations (Annis, Booth et al. 1963), new techniques (such as argon gas) have been developed, while older techniques – decompression chambers, strychnine, carbon monoxide inhalation – have fallen out of favor. Several techniques remain controversial, even in the face of multiple empirical assessments of their humaneness. The mid-1980s controversies around rodent decapitation have largely been eclipsed by concerns over the use of carbon dioxide (Carbone 1997; Carbone, Baumans et al. 2004; Conlee, Stephens et al. 2005). Meanwhile, there are essentially no published assessments of cervical dislocation in small rodents, though it is widely used and has been recommended by the AVMA panel since 1972 (Smith, Booth et al. 1972); I predict this will be the next big controversy in animal euthanasia circles.

As the AVMA Panel stresses, choice of euthanasia technique will depend on several factors, including species, the reason for euthanasia, and personnel factors. I want to stress that even the best euthanasia method can be botched horribly in the wrong hands. I think this "botch

factor" must be accounted for in any recommendations on how to kill animals. Though I find the arguments against carbon dioxide compelling, at most American institutions CO_2 remains the default euthanasia method for small rodents. One reason for its persistence is that it is relatively difficult, compared to physical techniques such as decapitation or cervical dislocation, or even to some anesthetic overdose techniques, to do it really badly. The commonest missteps with CO_2 euthanasia are overcrowding chambers with too many animals, and failing to assure that animals are truly dead; both of these are surmountable with adequate training and minimal oversight. A relatively foolproof but moderately painful technique could actually be preferable to one in which most animals experience no pain while a few experience severe pain.

Conclusion

The potential sources of distress in the animal laboratory are myriad. A tiny subset might be labeled "direct" – studies in which inescapable animal distress is itself the object of investigation. A larger fraction (though largely underreported) is those USDA Category E studies in which full treatment of distress would unacceptably compromise the data obtained. Many potential sources of distress can be anticipated and mitigated in protocol review. Others, including many husbandry and handling concerns, have nothing to do with the study at hand. Finally, some of the methods employed to manage or assess pain and distress (drugs prescribed, temperature and blood sampling methods) may themselves be sources of distress and must be applied judiciously.

With the range of species and experiments in modern laboratories, it is impossible to give more than this general outline here. Nonetheless, the concerns discussed here can serve as something of a checklist for preparing an animal care and use protocol that identifies most of the potential areas for distress (choice of animal, choice of housing, monitoring and treatment options, handling and manipulations, endpoint and euthanasia considerations) and prevents them before they arise.

Acknowledgment

Many thanks to David Takacs for helpful comments on this draft.

References

Animal and Plant Health Inspection Service, U. (1989). "Animal Welfare; Final rules." *Federal Register* 54(168): 36112 - 36163.

Animal and Plant Health Inspection Service, U. (1991). "9 CFR Part 3 Animal Welfare; Standards; Final rule." *Federal Register* 56(32): 6426 - 6505.

Animal Care Panel (1963). *Guide for Laboratory Animal Facilities and Care*. Washington, DC, Public Health Service.

Animal Welfare Institute (1953). *Basic Care of Experimental Animals*. New York, Animal Welfare Institute.

Annis, J. R., N. H. Booth, et al. (1963). "Report of the AVMA Panel on Euthanasia." Journal of the American Veterinary Medical Association 142(2): 162-170.

Beaver, B. V., W. Reed, et al. (2001). "2000 Report of the AVMA Panel on Euthanasia." Journal of the American Veterinary Medical Association 218(5): 669-96.

Broom, D. M. (1998). Animal welfare, coping. *Encyclopedia of Animal Rights and Welfare*. M. Bekoff and C. Meaney. Westport, CT, Greenwood Publishing Group: 58-60.

Burghardt, G. M. (1996). "Environmental enrichment or controlled deprivation." *Scientists Center for Animal Welfare Newsletter* 18(4): 3-10.

BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement (2003). "Refinement and reduction in production of genetically modified mice." *Laboratory Animals* 37 Suppl 1: S1-S49.

Carbone, L. (2004). *What Animals Want: Expertise and advocacy in laboratory animal welfare policy*. New York, Oxford.

Carbone, L., V. Baumans, et al. (2004). "Report of the workshop on euthanasia practices and guidelines (4th World Congress on Alternatives)." Alternatives to Laboratory Animals, 32 (Suppl 1B): 445-446.

Carbone, L., L. Guanzini, et al. (2003). "Adoption options for laboratory animals." *Lab Animal* 32(9): 37-41.

Carbone, L. G. (1997). "Death by decapitation: A case study of the scientific definition of animal welfare." *Society and Animals* 5(3): 239-256.

Close, B., K. Banister, et al. (1996). "Recommendations for euthanasia of experimental animals: Part 1." *Laboratory Animals* 30: 293-316.

Close, B., K. Banister, et al. (1997). "Recommendations for euthanasia of experimental animals: Part 2." *Laboratory Animals* 31: 1-32.

Committee on Dogs, I. o. L. A. R. (1994). *Dogs: Laboratory Animal Management*. Washington, D.C., National Academy Press.

Committee on Infectious Diseases of Mice and Rats, N. R. C. (1991). *Companion Guide to Infectious Diseases of Mice and Rats*. Washington, D. C., National Academy Press.

Committee on Laboratory Animal Housing, I. L. A. R. (1976). *Laboratory Animal Housing*, Hunt Valley, MD, National Academy of Sciences.

Conlee, K. M., M. L. Stephens, Rowan, A.N., and King, L.A. (2005). "Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats." *Laboratory Animals* 39(2): 137-61.

Crouse, S. J., E. R. Atwill, et al. (1995). "Soft surfaces: A factor in feline psychological well-being." *Contemporary Topics in Laboratory Animal Science* 34(6): 94-97.

Dodds, W. J. (1992). Bleeding disorders. *Handbook of Small Animal Practice*. R. V. Morgan. New York, Churchill Livingstone: 765-777.

Gordon, C. J. (1990). "Thermal biology of the laboratory rat." Physiology and Behavior 47(5): 963-91.

Gunn, D. and D. B. Morton (1993). The behaviour of singly-caged and group-housed laboratory rabbits. *Welfare and Science: Proceedings of the Fifth Symposium of the Federation of European Laboratory Animal Science Associations*, Brighton, UK, Royal Society of Medicine Press.

Hedenqvist, P., J. V. Roughan, et al. (2001). "Induction of anaesthesia with desflurane and isoflurane in the rabbit." *Laboratory Animals* 35(2): 172-9.

Held, S. D. E., R. J. Turner, et al. (1995). "Choices of laboratory rabbits for individual or group-housing." *Applied Animal Behaviour Science* 46: 81-91.

Hendriksen, C. F. M. and D. B. Morton, Eds. (1999). *Humane Endpoints in Animal Experiments for Biomedical Research*. London, Royal Society of Medicine Press.

Hume, C. W. (1957). The legal protection of laboratory animals. *The UFAW Handbook on the Care and Management of Laboratory Animals*. A. N. Worden and W. Lane-Petter. London, Universities Federation for Animal Welfare: 1-14.

Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. Washington DC, National Academy Press.

Ivani, G., C. Vercellino, et al. (2003). "Ketamine: a new look to an old drug." Minerva Anestesiologica 69(5): 468-71.

Kalagassy, E., L. G. Carbone, et al. (1999). "Effect of castration on rabbits housed in littermate pairs." *Journal of Applied Animal Welfare Science* 2(2): 111-121.

Kennedy, R. M. and J. D. Luhmann (2001). "Pharmacological management of pain and anxiety during emergency procedures in children." *Paediatric Drugs* 3(5): 337-54.

Love, J. A. (1994). "Group housing: Meeting the physical and social needs of the laboratory rabbit." *Laboratory Animal Science* 44(1): 5-11.

Mandriota, S. J., L. Jussila, et al. (2001). "Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis." *The EMBO Journal* 20(4): 672-82.

Martin, B. J., B. E. Crook, et al. (1994). "Cage height preferences of laboratory rats." *Contemporary Topics in Laboratory Animal Medicine* 33(4): A-8.

McMillan, F. D. (1999). "Effects of human contact on animal health and well-being." Journal of the American Veterinary Medical Association 215(11): 1592-1598.

Mench, J. A. (1994). "Environmental enrichment and exploration." *Lab Animal* 23(2): 38-41.

Merskey, H. and N. Bogduk, Eds. (1994). Classification of Chronic Pain. Seattle, IASP Press.

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. *The Biology of Animal Stress: Basic principles and implications for animal welfare*. G. P. Moberg and J. A. Mench. Wallingford UK, CABI Publishing: 1-21.

Morton, D. B. (1998). Dystress. *Encyclopedia of Animal Rights and Welfare*. M. Bekoff and C. Meaney. Westport, CT, Greenwood Publishing Group: 139-140.

Morton, D. B. and et. al. (1993). "Refinements in rabbit husbandry. Second report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement." *Laboratory Animals* 27: 301-329.

Office of Protection from Research Risks (1986). *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Bethesda, Md., National Institutes of Health.

Overall, K. L. (1997). *Clinical Behavioral Medicine for Small Animals*. St. Louis, Mosby.

Poole, T., Ed. (1999). *The UFAW Handbook on the Care and Management of Laboratory Animals*. Malden, MA., Blackwell Science.

Quimby, F. (2002). Animal models in biomedical research. *Laboratory Animal Medicine*. J. G. Fox, L. C. Anderson, F. M. Loew and F. Quimby. New York, Academic Press: 1185-1219.

Raje, S. and K. L. Stewart (1997). "Group housing for male New Zealand White rabbits." *Lab Animal* 26(4): 36-38.

Reinhardt, V. (1991). "Impact of venipuncture on physiological research conducted in conscious macaques." *Journal of Experimental Animal Science* 34(5-6): 212-7.

Reinhardt, V. and A. Reinhardt, Eds. (2002). *Comfortable Quarters for Laboratory Animals*. Washington, D. C., Animal Welfare Institute.

Ruiven, R. v., G. W. Meijer, et al. (1996). "Adaptation period of laboratory animals after transport: a review." Scandinavian Journal of Laboratory Animal Science 23(4): 185-190.

Russell, W. M. S. and R. L. Burch (1959). *The Principles of Humane Experimental Technique*. London, Methuen & Co. Ltd.

Smith, C. R., N. H. Booth, et al. (1972). "Report of the AVMA Panel on Euthanasia." Journal of the American Veterinary Medical Association 160(5): 761-772.

SoRelle, R. (2004). "Rofecoxib use increases acute myocardial infarction risk." Circulation 109(17): e9039-40.

Thompson, A. C., M. B. Kristal, et al. (2004). "Analgesic efficacy of orally administered buprenorphine in rats: methodologic considerations." Comparative Medicine 54(3): 293-300.

Toth, L. A. (1997). "The moribund state as an experimental endpoint." *Contemporary Topics in Laboratory Animal Science* 36(3): 44-48.

Turner, R. J., S. D. E. Held, et al. (1997). "An immunological assessment of group-housed rabbits." *Laboratory Animals* 31: 362-372.

United States Congress (1985). *Food Security Act of 1985 (Public Law 99-198). Title XVII, Subtitle F - Animal Welfare*.

United States Congress (1985). Subtitle F - Animal Welfare. *United States Code Congressional and Administrative News, 99th Congress - First Session, 1985*. St. Paul, Minn, West Publishing Co. 3: 2518-2524.

Wolfle, T. L. (2000). Understanding the role of stress in animal welfare: practical considerations. *The Biology of Animal Stress: Basic principles and implications for animal welfare*. G. P. Moberg and J. A. Mench. Wallingford UK, CABI Publishing: 355-368.