AATEX **14**, Special Issue, 21-27
Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences
August 21-25, 2007, Tokyo, Japan

Alternative research and practice supported by international veterinary professionals

Judy MacArthur Clark

President, International Association of Colleges of Laboratory Animal Medicine
96 Chester Street, Chester, NH 03036, USA
judy@solomon-foundation.org

Abstract
Welfare in a research setting has, over recent years, been increasingly influenced by the implementation of the Three Rs – Replacement, Reduction and Refinement. Social perceptions of welfare throughout the world have demanded greater awareness of welfare which supports this development. In the early to mid eighties, several exposées occurred which resulted in great public concern about animal research, and consequential changes to national legislation on this topic were implemented in many developed countries of the world. Two key issues pervade almost all of that legislation – ethical review of proposed procedures, and veterinary oversight. Thus veterinary profession plays a significant role in the effective implementation of the Three Rs, particularly Refinement. Additionally, it is imperative that we gain a greater understanding of two further aspects of our use of animals in research; first the environmental needs of our research animals, and second, an understanding of their affective states when exposed to a range of experimental interventions – and how best we can address those states.

Keywords: animal welfare, refinement, veterinarians, ethical review, veterinary oversight

Introduction

When we think of animal welfare, we often focus on discrete groups of animals – in zoos, on farms, in laboratories and so on. However of greater importance may be to make the connections between these different groups of animals so that we can learn from one field to another. What can techniques used in farm animal welfare research tell us about laboratory animal welfare? How can we make use of the resources directed to the welfare of one group of animals to help another group of animals? This is a potential future for animal welfare - sharing resources and creating dialogue between parties who at this time do not meet and yet have very common interests for the benefit of animals. And veterinarians occupy an important position in realising these connections.

The three Rs of Russell & Burch

As I look back through recent decades, I see missed opportunities when these connections might have borne fruit. In 1974, I joined UFAW (Universities Federation for Animal Welfare) and soon became aware of the Principles of Humane Experimental Technique by William Russell and Rex Burch, the product of a project sponsored by UFAW in the 1950s. However fifteen years after its publication, what seemed to me to be a very important piece of work was all but ignored by the scientific and animal welfare community.

Russell and Burch started their UFAW project in 1954 and interviewed more than 100 active UK scientists in preparing their report. They first described the principles of "Replacement, Reduction and Refinement", now known as the Three Rs, at a conference in London in 1957. Two years later they published their remarkable book (Russell & Burch, 1959).

Admittedly the text is not an easy read – you can access it on the Johns Hopkins Center for Alternatives website (http://altweb.jhsph.edu/publications/humane_exp/het-toc.htm) – but the concepts it contains foresaw the direction in which animal welfare would move during the next half century and beyond. However it is important to realise that their Three Rs can be applied not just to research animals, but also in all other fields of animal welfare, particularly the last of the three - Refinement. For example, as chair of the UK Farm Animal Welfare Council, I learned that even small steps in refinement of farming practices could lead ultimately to better welfare systems without destroying profitability (and the inevitable accusation of "exporting" animal welfare problems).

Changing consumer perceptions

However of equal importance in driving such a trend is the perception of the marketplace (consumers, and public concern for animal welfare). Of course, many new concepts which change our view of life, art and science are a result of the confluence of several diverse ideas at a particularly fruitful time. In this regard, attitudes towards animal welfare broadly have changed in recent years in many parts of the world. I do not measure this in terms of a rise in animal activism but rather a rise in public concern for animals.

In a recent survey of American attitudes to animal welfare generally (Bennett et al, 2002), 76% of respondents were concerned that farm animals might be mistreated or suffer in current food production systems. And a remarkable 83% of respondents agreed with the statement that "it is wrong to cause farm animals any pain, injury or stress". One may view these responses as naïve reflections of the public understanding of modern farming production. But they can also be interpreted as a wake-up call for farmers and farming practices.

Likewise, in the UK, we see increased criticism of current standards of animal care with 67% feeling that levels of welfare in farming are poor, and 76% seeking more information from food retailers (European Commission, 2005). While we know that these views are not necessarily reflected in the purchasing decisions of consumers, they nevertheless reflect a level of concern which has not been seen previously.

A study in Australia (Franklin, 2007) has attempted to understand this changing attitude to our relationship with animals. When asked whether they were more or less likely to perform certain activities compared with a few years ago, 52% were more likely to watch wildlife programmes, 30% more likely to donate to animal charities, 48% more likely to buy free-range eggs, and 37% less likely to buy products which had been tested on animals. An interesting 55% of those polled felt that animals have the same moral rights as humans.

Issues and exposés

In 1982, I was awarded a Winston Churchill Fellowship to visit North America. The main purpose of my trip was to study postgraduate training of veterinarians in laboratory animal medicine. However recent events in Silver Springs, Maryland, persuaded me to also explore the growing impact of the animal rights movement in North America. At the time I believed the two issues to be relatively unconnected and I reported on them separately. However, over time the close connection between these two fields of investigation has become increasingly apparent to me and to others.

In May 1981, Alex Pacheco, then a politics student at George Washington University, volunteered for a job with Dr Edward Taub at the Institute for Behavioral Research in Silver Springs. Only a few months earlier, Pacheco and Ingrid Newkirk had set up PeTA – People for the Ethical Treatment of Animals. While Taub was away on vacation, Pacheco took photos of the 15 or so deafferented monkeys. Most animals had both sensory and motor nerves of one arm severed. Pacheco also invited several veterinarians into the facility to experience the conditions in which the animals lived. Finally he arranged for State Police to raid the facility under the Prevention of Cruelty to Animals law of the State of Maryland.

Taub was convicted on six counts of animal cruelty but these were set aside by the Court of Appeal since the issue was judged to be a federal matter since Taub received federal funding. The Office for Protection from Research Risks (OPRR) then investigated the apparent serious non-compliance with Public Health Service (PHS) policy but was never able to reach a firm conclusion. On the one hand, it appeared that Taub may have been set up by PeTA. On the other, it was clear from Taub's records that the animals had not received veterinary care for a period of several years in spite of being deafferented which would require more specialized care than most primates. This constituted a serious violation of the PHS policy and Taub's grant was suspended until he could show full compliance with the Guide. The laboratory was never restored and the animals remained in the care of NIH (National Institutes of Health) in spite of efforts by PeTA to gain custody of them. Overall, the "Case of the Silver Springs Monkeys" lasted over ten years in the media until the last of the monkeys was euthanized.

Unnecessary Fuss was a 26 minute video produced by PeTA two years later arising from a break-in to the Head Injury Laboratory at the University of Pennsylvania by members of the ALF (Animal Liberation Front). At the time of the break-in, the ALF stole over 60 hours of audio and videotape recorded by the staff at the laboratory. They then gave the stolen tapes to PeTA who edited the tapes, provided a voice over commentary, and issued the recording to schools, the media, and Congress. To say that Congress and members of the general public were shocked would be an understatement. The cruelty and general disregard for the animals seemed to be appalling and there was public outcry.

When OPRR insisted on seeing the entire taped material in order to investigate the claims, it became apparent that Unnecessary Fuss presented the case history of only one of approximately 150 animals. It used clever editing and inaccurate voice-over to mislead the viewer into believing that the inhumane

treatment on the film was repeated numerous times. However, despite the film's overstatement, OPRR found serious violations of the ILAR Guide for Laboratory Animal Facilities and Care (National Research Council, 1978). In particular, veterinary and post-trauma care was inadequate and surgical hygiene was poor.

Britches was the name of an infant stump-tailed macaque born into a Californian breeding colony in 1985. He was removed from his mother at birth and his eyelids sewn shut as part of a three year maternal and sensory deprivation study under Dr David Warren at the University of California at Riverside. At five weeks of age, Britches was removed from the facility during a raid by the ALF and his "rescue" was videotaped. PeTA subsequently released the videotape and there was general condemnation of the experiments, not only by fellow scientists but also by the American Council of the Blind. Eight of the seventeen studies interrupted were never restarted, and the University stopped allowing baby monkeys eyes to be sewn shut.

These are all disturbing events which occurred between 1981 and 1985. But let me be clear that I am not confusing these exposés with more recent activities of people such as Jerry Vlasak in this arena. Vlasak, who is a trauma surgeon working in California and presumably is therefore daily making use of many products of animal research to save human lives, has publicly made inflammatory comments that "for five, ten or fifteen human lives we could save one, two or ten million nonhuman lives". This lead to the UK Government refusing to allow Vlasak to enter the country, and has caused similar stirs in the US Congress. I believe that the examples of exposés in the 1980s to which I have referred differ significantly from the activities of Vlasak in this current decade. And it is this difference which makes them worthy of remembering today.

The particular three events I have described have the following elements in common. Firstly, they were all exposed by a raid or infiltration by animal rights activists but, upon investigation, there was also severe criticism of the practices not just by the activists and the media, but also by peer scientists and veterinarians. Secondly, there was an overall lack of internal oversight of the procedures which were being conducted within each institution. Finally, there was an obvious lack of veterinary care even though the animals involved were some of those perhaps most in need of such care.

It is therefore not surprising that, in 1985, ILAR published a new edition of the Guide, this time entitled the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985), and a new PHS Policy (US Public Health Service, 1985) was promulgated which required each institution in receipt of federal grants to appoint an Institutional Official, an IACUC (Institutional Animal Care and Use Committee) and an Attending Veterinarian. Prospective and ongoing protocol review by the IACUC, as well as semiannual inspections of the facilities, became part of the norm. At the same time on the other side of the Atlantic Ocean, significant changes were also taking place. In this same time period we saw the conclusion of negotiations leading to the Council of Europe Convention for the protection of research animals (Council of Europe, 1985), the passage of the Animals (Scientific Procedures) Act in the UK (UK Home Office, 1986), and the approval of the EU Directive 86/609 (European Commission, 1986) which is largely based upon the Council of Europe Convention. All of these new frameworks identified the importance of veterinary oversight in assuring the welfare of animals in research.

But it is also interesting to reflect on where the key players in these exposés are now, and what has been their contribution to medical research. Edward Taub has pioneered a novel treatment for stroke called Constraint-induced Movement Therapy, based largely upon his studies in primates. As a result, thousands of stroke patients have a significantly better quality of life than would otherwise have been the case. Thomas Gennarelli practises neurosurgery in Wisconsin and has built a reputation in the treatment of traumatic brain injury. As a result of his work, improved safety helmets have been designed for military pilots but also for participants in high risk sports such as mountain bike racing. Many young people around the world owe their lives to his work. David Warren is still at UC Riverside as an emeritus professor. His work has lead to a greater understanding of cognitive development in visually impaired children.

The importance of ethical review and veterinary oversight

So does this mean that what these individuals were doing in the 1980s is excusable? Of course the answer is emphatically no. Because there is no doubt that they could have made the same advances in a more humane manner which respected the welfare and needs of the animals. They could have sought the advice of colleagues such as veterinarians and experienced animal care staff to ensure that their science was advanced in the most humane way possible. However, it is also important that such advisers keep in mind the importance of scientific discovery. By developing an open dialogue, everyone involved can ensure that the needs of the science are appropriately balanced against the needs of the animals. This includes scientists and veterinary staff, but also animal care staff who observe animals on a daily basis. Together, and as a team, they can ensure that the 3Rs are applied to proposed and on-going studies as rigorously as possible.

Which brings us back to where we started – with Russell and Burch. Because much of the impact of ethical review and veterinary oversight is seen in the third of the Three Rs – Refinement. It is important that scientific protocols are reviewed by scientific peers – those who can understand the science and the need for the proposed studies. But it is equally important that this review is balanced with the interests of the animals - understanding what they will experience during the protocol. It is therefore not surprising that these two components, protocol review and veterinary oversight, are viewed as essential basic elements in most laboratories in the Western world, either because the regulations demand this (e.g. in the US, the requirement for an IACUC and an Attending Veterinarian; in the UK, the mandatory Ethical Review Process and the Named Veterinary Surgeon; and in Europe, the new proposals being considered to replace 86/609/EEC), or because institutes and corporations have simply decided that this is the right thing to do. Furthermore, experience shows it is the interplay between the two, the Veterinarian having responsibilities which he or she fulfills on behalf of the Committee, that allows the system to work so effectively in a balanced manner.

Additional benefits of such oversight are regular review of the physical facilities within which animal research is conducted, the creation of an avenue through which animal care staff and others can make their views known, and the opportunity for the Institute or Company to set its own culture which may sometimes be more stringent than the legal environment in which it operates. Perhaps of equal importance is the presence of lay members on protocol review committees. This opens up the dialogue to improve public understanding of the issues associated with animal research and the strenuous attempts which are made to minimise pain or distress caused to subjects of that research. In an ideal world, this dialogue with the public would be much more open and widespread and, indeed, many scientists have stepped into the public domain to explain their work and its impact. However it has to be recognised that the threatening behavior of animal rights activists encourages an atmosphere in which such openness may be considered foolhardy and has lead to personal attacks.

The role of international veterinary professionals

So how can international veterinary professionals make these processes of protocol review and oversight work even better? This is where Colleges of Laboratory Animal Medicine can play a critical role. Their remit is to raise the standards of veterinary training in laboratory animal medicine, and to provide a core group of highly experienced individuals who have proven their expertise through examinations or similar evidence. IACLAM (the International Association of Colleges of Laboratory Animal Medicine) was established in 2005 to represent all Colleges on a global platform (MacArthur Clark, 2007a). It promotes the welfare and responsible use of laboratory animals by certifying veterinary specialists through the individual Colleges and by serving as a research partner, especially in promoting refinements. It also acts as a unified voice of veterinary expertise, disseminating information relevant to the field in international forums. Colleges currently exist in North America, Europe, Japan and Korea but the role of IACLAM spreads much more widely in encouraging and supporting training in laboratory animal medicine in regions where there is as yet no College and where the speciality may, as yet, be fairly embryonic.

I believe there are three areas where veterinarians have much to offer in refinement to improve animal welfare. The first, assuring good health and functioning, can be assumed to be the direct responsibility of the Clinical Veterinarian and I will spend no further time considering it in this presentation.

Understanding environmental needs

The second relates to us understanding the environmental needs of our animals and how we can best address the most necessary of those needs. At one end of the spectrum we can consider chimpanzees and their complex needs. When you visit sanctuaries for retired research chimpanzee such as Chimp Haven in Louisiana, you can't help but be impressed how like humans these animals are. I have worked with other primate species; but in the chimpanzee I experience another order of social complexity, communication skills, organizational structures, and longevity which is manifested remarkably in an environment which approximates to that in the wild. It is hard to justify the containment of these animals in anything less than a semi-natural environment.

Roger Fouts is responsible for a unique family of chimpanzees at Central Washington University's Chimpanzee and Human Communication Institute. These chimps have learned American Sign Language and they use the signs in conversations with each other and with their human companions. Roger believes strongly that good empirical research must be humane which entails developing ways to improve their living conditions to meet their physical and psychological needs. I particularly like a quote taken from his book (Fouts & Mills, 1998) in which he describes the chimpanzee as a "highly intelligent, co-operative, and violent primate who nurtures family bonds, adopts orphans, mourns the death of mothers, practises self-medication, struggles for power, and wages war". How like our own human condition.

But what about other species more commonly

found in our laboratories such as rats. Georgia Mason of the University of Guelph mounts persuasive arguments why we should consider rats as carefully as primates in relation to their needs. As evidence, we know that rats can tell humans apart (Davis et al, 1997), rats can count (Suzuki & Kobayashi, 2000), rats have memories that almost certainly feel like ours (Babb & Crystal, 2006), rats probably dream (Ji & Wilson, 2007), rats understand cause and effect (Blaisdell et al, 2006) and rats understand their own state of knowledge (Foote & Crystal, 2007).

So does this mean we should provide an environment for rats which is close to their natural environment? As William Russell so astutely noted "Captive animals usually 'know what is good for them', and our chief concern must usually be to provide them with the essential components of the environment from which we have removed them. ...Domesticated animals, however, have lost many of their original responses, and suffered disruption of a formerly well-organized and dove-tailed behaviour system, in connection with their long history in a new kind of environment, one in which many of their needs may be supplied by man... We have often to supplement their behaviour, for we are now an essential part of their world" (Russell, 1956).

What may be most important therefore is to learn what is most needed by a domesticated animal and to then focus on providing for that need in its environment. Here we can learn from other fields of welfare research. Ian Duncan pioneered the techniques of preference testing to understand motivational behaviour in chickens (Duncan, 1992). He was able to show quite subtle features such as the strength of motivation a chicken has for a nest immediately prior to laying an egg; a motivation which is not present at other times. A total ethogram of the chicken would probably have not picked up this short term motivation, but asking the chicken to express its preference over time identified this powerful driver. He clearly demonstrated that, in order for results of preference tests to be properly interpreted, they should be followed up with appropriate tests to measure the strength of preference.

Preference testing has also been used more recently with laboratory rats, for example to evaluate types of housing conditions (Bloom et al, 1995) and nesting materials (Van de Weerd et al, 1998). However it remains important to interpret results with caution unless the strength of the preference has also been tested. For example, we have used the technique recently with laboratory mice to determine preference for different concentrations of environmental ammonia (Green et al, 2008). The results showed strong preference for upper compartments in a two-tier housing model, but with no clear preference for or against ammonia levels ranging from zero to over 100ppm. We used access to food to determine strength of preference.

From these and other experiments, we can try to devise living conditions which approximate to at least some of the basic needs of each species. We can use preference and other testing to allow species to inform us of what they need most, and we can enrich the environment to offer critical elements which meet these most important needs. There is no doubt that good quality research is needed to better understand the specific needs and preferences, and veterinary professionals should play a role, not just in this research but also in implementing the outcomes both in regulations and guidelines, and in everyday practices in research animal facilities.

Managing affective states

The third area where veterinarians can play an important role in refinement is in managing the affective states of animals, for example, by recognizing when they experience distress or pain. Elsewhere, I have discussed the importance of non-pain distress when it occurs before, during and after research procedures (MacArthur Clark, 2007b). In defining what we mean by pain, we can do no better than the 1979 definition of the International Association for the Study of Pain – "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (Merskey et al, 1979) together with the US Interagency Research Animal Committee (IRAC) advice in 1985 that "unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." (IRAC, 1985).

We use a range of methods to recognize and assess pain and distress. Firstly, we use clinical impressions including physical appearance, response to palpation, and behavioural changes. Secondly, we can use Pain Scoring Systems which might involve numerical rating or visual analogue scales. Thirdly we can measure physiological responses such as heart rate and blood pressure. We can also measure autonomic responses such as endocrine changes, e.g. in cortisol levels. Finally, we can use neurophysiological methods such as electroencephalograms and measurements of cortical activity. No one method alone can be an absolute measure. The role which a veterinarian can play is to encourage use of several methods with correlation of all the data to give an accurate and unbiased picture.

Scoring systems can be especially useful where a particular procedure is performed on numerous occasions with diverse outcomes in terms of pain and distress for the subject animals. Since the pioneering work of Morton and Griffiths (Morton & Griffiths, 1985), many other systems have been developed. The

guiding principles appear to be to keep the system as simple as possible and relevant to the specific procedures involved. A small number of variables should be determined and a system of weighting these variables may be considered. It is important to avoid variation between observers by describing the variables and score categories, and by giving training in their application. Generally two or three point scales are preferable and the scoring system should be piloted if possible. Data derived from such simple scoring systems can be very persuasive in justifying the use of analgesia or other methods to minimize pain or distress.

Finally let me consider recent work which will have a profound impact on the welfare of literally millions of research animals, particularly rodents. Carbon dioxide (CO2) has been used for many years as a euthanasia agent for a number of species. Recently, the humaneness of this approach has been challenged and studies to compare the effect of gradual-fill of the euthanasia chamber compared with pre-fill have been performed. On balance, gradual-fill takes longer but appears to be more humane though neither method is free from distress (Neil & Weary, 2006). The outcome has been to promote a pragmatic solution since no other practicable gaseous method is currently available. Equipment to reliably deliver the gradual-fill has been developed and, meanwhile, research into more humane methods of gaseous euthanasia is underway (Hawkins et al, 2006). An important role for veterinarians is to not only promote and participate in this research, but also to ensure broad awareness of these finding and their application.

Conclusions

In conclusion, I would summarise the theme of my presentation with the phrase "Balance through Dialogue". By encouraging dialogue between all parties concerned with the use of animals, including those using animals for the benefit of humankind (e.g. for food production or medical research) and those promoting animal protection, we can aim to achieve the right balance between public interest and concern, and the justifiable need for animal use. We need also to have this dialogue between the diverse fields of animal welfare - on farms, in zoos and in laboratories - to share ideas and experiences and to learn from each other's research. It is also important to ensure balance between what research data may be telling us and how we implement those data, whether this is in terms of improving housing conditions, improving methods of euthanasia or humane slaughter, improving transport conditions or some other aspect important to animal welfare. Development of this dialogue is perhaps one of the most important current priorities for improved welfare through refinement. International veterinary professionals, who often occupy a uniquely informed, independent, neutral and respected position in their field, should be leading and encouraging others in this effort.

References

Russell, WMS. & Burch, R. (1959). Principles of Humane Experimental Technique. UFAW, London. Also http://altweb.jhsph.edu/publications/humane_exp/het-toc.htm

Bennett, R. M., Anderson, J., Blaney, R.J.P., (2002) Moral intensity and willingness to pay concerning farm animal welfare issues and the implications for agricultural policy. J. Agric. Econ. 15, 187–202.

European Commission, (2005) http://europa.eu.int/comm/food/consultations/internet_consultation/united_kingdom.pdf

Franklin, A. (2007) Human-Nonhuman Animal Relationships in Australia: An Overview of Results from the First National Survey and Follow-up Case Studies 2000-2004. Society and Animals, 15, 7-27.

National Research Council Institute of Laboratory Animal Resources, (1978) Guide for Laboratory Animal Facilities and Care, 5th edition. National Academy Press: Washington DC.

National Research Council Institute of Laboratory Animal Resources, (1985) Guide for the Care and Use of Laboratory Animals, 6th edition. National Academy Press: Washington DC.

US Public Health Service,(1985) Public Health Service Policy on the Humane Care and Use of Laboratory Animals. US Dept of Health & Human Services 28pp [PL-99-158, Health Research Extension Act, 1985]: Washington DC.

Council of Europe, (1985) Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific purposes (ETS 123). Strasbourg.

UK Home Office, (1986) Animals (Scientific Procedures) Act. HMSO; London.

European Commission, (1986) EC Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific purposes (86/609/EEC). Brussels.

MacArthur Clark, JA., (2007a) IACLAM; A Global Vision for Laboratory Animal Medicine. in Proceedings of the 6th World Conference on Alternatives and Animal Use in the Life Sciences, Tokyo, Japan, August 2007, 735-737.

Fouts, R. & Mills, ST., (1998) In Next of Kin: my conversations with chimpanzees. Avon Books Inc, New York.

Davis, H., Taylor, AA. & Norris, C. (1997) Preference for familiar humans by rats. Psychonomic Bulletin & Review, 4(1), 118-120.

Suzuki, K. & Kobayashi, T. (2000) Numerical competence in rats (Rattus norvegicus): Davis and Bradford Extended. J. Comp. Psychology, 114, 73-85.

Babb, S. & Crystal, J. (2006) Episodic-like memory in the rat. Current Biology, 16 (13), 1317-1321.

Ji, D. & Wilson, MA. (2007) Coordinated memory replay in the visual cortex and hippocampus during sleep. Nature Neuroscience, 10, 100-107.

Blaisdell, AP., Sawa, K., Leising, KJ. & Waldman, MR. (2006) Causal reasoning in rats. Science, 311, (5763) 1020-1022.

Foote, A. & Crystal, J. (2007) Metacognition in the rat. Current Biology, 17(6), 551-555.

Russell, W. M. S. (1956) On Misunderstanding Animals. UFAW Courier, 12:19-35.

Duncan, IJ. (1992) Measuring preferences and the strength of preferences. Poult. Sci., 71(4), 658-663.

Bloom, HJM., Van Tintelen, G., Baumans, V., Van Den Broek, J. & Beynen, AC. (1995) Development and application of a preference test system to evaluate housing conditions for laboratory rats. Appl. Anim. Behav. Sci. 43, 279-290.

Van de Weerd, HA. Van Loo, PLP. Van Zutphen, LFM, Koolhaas, JM & Baumans, V. (1998) Strength of preference for nesting material as environmental enrichment for laboratory mice. Appl. Anim. Behav. Sci. 55, 369-382.

Green, AR., Wathes, CM., Demers, TGM., MacArthur Clark, JA. & Xin, H. (2008) Development and Application of a novel environmental preference chamber for assessing responses of laboratory mice to atmospheric ammonia. J. Am. Ass. Lab. Anim. Sci. In Press.

MacArthur Clark, JA., (2007b) Does Distress Matter? Proceedings of the 6th World Conference on Alternatives and Animal Use in the Life Sciences, Tokyo, Japan, August 2007, 93-95.

Merskey, H., Albe-Fessard, DG., Carmon, A., Dubner, R., Kerr, FWL., Lindblom, U., Mumford, JM., Nathan, PW. & Noordenbos, W. (1979) Pain terms: a list with definitions and notes on usage. Pain, 6, 249-252.

IRAC (Interagency Research Animal Committee), (1985) U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Federal Register, May 20, 1985. Office of Science and Technology Policy:Washington DC.

Morton, DB. & Griffiths, PH. (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet. Rec. 116, 431-436.

Niel, L. & Weary, D. (2006) Behavioural responses of rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. Appl. Anim. Behav. Sci. 100, 295-308.

Hawkins, P., Playle, L., Golledge, H., Leach, M., Banzett, R., Coenen, A., Cooper, J., Danneman, P., Flecknell, P. & Kirkden, R. (2006) Newcastle concensus meeting on carbon dioxide euthanasia of laboratory animals. Animal Technology and Welfare, 5(3), 125-134.