Theme IV:
Animal Welfare for Refinement and High Quality Science

Session summaries for Theme IV

Session IV-1

Indicators of Animal Welfare to Implement Refinement

Co-Chairs: David Mellor, Massey University, New Zealand
Marilyn Brown, Charles River, USA

This session began with four review-like evaluative presentations. The purpose of these presentations was to encourage fresh thinking about the theoretical background of Refinement and the range of potential animal experiences, as well as the limitations in understanding and the types of impact assessment that, when considered together, enhance the scope and effectiveness of Refinement applications. These papers were followed by two accounts of specific research studies that provide cautionary advice on the context-specificity and interpretation of particular behavioral indices of negative and positive animal experiences.

The major theme of the first paper, entitled *Affective states and the assessment of laboratory-induced animal welfare impacts* (by **David Mellor**), was that there is significant scope for refinement strategies to become simultaneously more focused and expanded in their application. This can be achieved by recognizing, first, that in view of the many different types of pain animals may experience and their diverse sources, pain is a good initial focus for refinement strategies; second, that the catchall term "distress" includes numerous specific types of negative experience, each of which may be targeted; and third, that various factors can have negative impacts on animals by preventing positive experiences, so that Refinements should also be directed towards promoting positive experiences.

The second paper, entitled *The sensitivity of animals and application of the Three Rs* (by **Emily Patterson-Kane**), explored the notion that we implicitly restrict our thinking about the sensory and other capabilities of animals only to those capabilities we ourselves possess. We thereby greatly underestimate the sensitivity of animals to environmental cues, human presence and, possibly, to human intentions, and this means that quite subtle Refinement requirements and opportunities may be missed.

The next paper, entitled *Complementary roles for systematic analytical evaluation and qualitative whole animal profiling in welfare assessment for Three Rs application* (by **Ngaio Beausoleil** and **David Mellor**), explored the key features of two approaches to assessing the welfare status of animals. It was suggested that combining both approaches in assessments of laboratory animal welfare will facilitate more thorough consideration of welfare status, enabling immediate or future Three Rs mitigation strategies to be identified, applied, and evaluated.

The fourth paper, entitled *Reliance on behavior as a metric of animal welfare* (by **Kathryn Bayne**), then drew attention to limitations in the use of behavior alone to gauge the efficacy of Refinement strategies. It highlighted factors such as timing of observations in the diurnal cycle, the skills of observers, and individual differences in response. Yet, it was argued that, with certain safeguards, behavior could be used validly to assess an animal's state.

The first of the experimental reports, entitled *Behavior changes during rat euthanasia may be a poor indicator of aversion* (by **Joanna Makowska** and **Dan Weary**), outlined behavioral responses that suggested the use of isoflurane anaesthesia was more aversive than CO_2 use, in contrast to extensive evidence from approach-avoidance, avoidance-avoidance, and total dwelling time studies that indicated the opposite. Rethinking this initial interpretation led to the conclusion that the gross behaviors observed reflected the stage of excitation during the induction of isoflurane anaesthesia, not aversion.

The second experimental report, entitled *Impact of simple environmental improvements on affective behavior, physiology and immune system reactivity of C57BL/6 and BALB/c mice* (by **Pat Turner** and colleagues), dealt with the impact of various inexpensive cage improvements on a range of functional indices of general health and stress levels, as well as behaviors reflective of affective state. The outcome was that the cage improvements did not significantly alter many physiological parameters within a particular age group or sex but appeared to improve the overall well-being of the rats, in particular through a reduction in aggressive interactions between cage mates.

Session IV-2

Farm Animal Research and the Three Rs

Co-Chairs: Marina von Keyserlingk, University of British Columbia, Canada
Dorte Bratbo Sorensen, University of Copenhagen, Denmark

Open minded communication and education between the public, the scientist, the animal care-giver and animal ethics committees is an important part of ensuring a good life for all animals. People working with laboratory animals have traditionally been comprised of highly educated individuals who tend to be very uniform in their view of how animals should be housed and cared for. In contrast, the number and type of individuals working with farm animals is much broader, including a diversity of views, values, and traditions. Including the farm animal perspective when evaluating the welfare of animals used in science broadens the discourse and provides other avenues for bridging between the science, its critics and the public. Ideally, a good life for a farm animal used in science (and on farms) acknowledges affective states, natural living and basic health. Animal welfare science attempts to address all three types of concerns by identifying problems and providing solutions that take all concerns into consideration.

Presentations were made by Dr **David Fraser**, Dr **David Mellor**, Dr **Dorte B. Sørensen**, and Dr **Patricia Turner**.

Session IV-3

Wildlife Science and the Three Rs

Co-Chairs: Marc Cattet, University of Saskatchewan, Canada
Chris Darimont, University of California, Santa Cruz, USA

This session brought together a diverse set of speakers that explored how the three Rs are and can be applied to wildlife science. Typically applied to animals under direct, full-time stewardship by humans (e.g., farm, laboratory and human companion animals), these ethical guidelines for humane treatment are increasingly being considered by wildlife scientists, policy makers, and the public. Application in this new domain provides both challenges and opportunities, which served as a theme that united all presentations.

The session started with a broad introduction. **Chris Darimont** opened by outlining how animal welfare, the scholarly domain of the 3 Rs, is being increasingly considered in the context of wildlife science. This includes recognition that the 3 Rs can provide ethical guidance towards reducing potentially negative welfare impacts wrought by invasive scientific tools. Mark Wallace, the session's invited speaker, explored in depth the ethical concerns of conducting wildlife research. Drawing on diverse ethical frameworks (Animal Ethics, Environmental Ethics, and Research Ethics), he synthesized their inadequacies as applied to the 3 Rs as mitigation strategies. Mark then made the case for a new ethical framework, termed "ecological ethics" which, along with the 3 Rs, would include a fourth R for "refusal." This last R would be applied in situations where the knowledge gained by the proposed research is likely to be outweighed by the harm done to the study animal.

Vivek Sharma's talk on *refinement* identified alternative non-invasive molecular techniques for use in the study of biosystematics that his team has advanced in their study on amphibian diversity in India. Although some handling of animals is required, the sampling is relatively painless and does not require killing animals, making it not only more ethical but more appropriate for working with potentially endangered species. **Ngaio Beausoleil** also presented on refinement, with a focus on refining lethal pest control in a New Zealand system. Ngaio and her team are conducting welfare assessments for various methods of control to determine how humane current techniques are, arguing that a ranking system is needed for the refinement process. This systematic approach has enabled the ranking of control methods and the identification of specific physical impacts caused by different methods, as well as highlighting future research needs.

Michelle Hiltz presented the results of computer simulation that can *replace* animal use in the development of humane kill traps used primarily for the subsistence and commercial harvesting of fur-bearing mammals. Drawing on approximately 25 years of data that involved: 1) mechanical evaluations of traps and; 2) compound testing, which involves testing on live animals in a field enclosure to collect additional data on animal behavior, time to loss of consciousness, time to death, signs of pain and distress, *etc*.; Michelle built predictive models to evaluate how humane different lethal traps are. As of 2011, computer simulations are now so advanced that step 2) is being omitted for many types of kill traps. Simulation results have been cross-validated with previous evaluations that used compound testing. To date, these models have reduced the number of animals tested by more than 1,400 and have resulted in a savings of over $ 4 million.

Transcending the 3 Rs, **Marc Cattet** presented results that revealed significant biases that invasive wildlife research techniques can impose on scientific inquiry. Marc first shared his findings from a long-term grizzly bear study that showed that body condition was significantly poorer for individuals captured multiple times compared with those captured once. In a polar bear study, he next showed that capture number and frequency contributed significantly to explaining body condition – so much so that their novel inclusion in models suggested that current indices of sea-ice availability (e.g., mean date of ice break-up) were less important in explaining body condition. Such findings highlight how the 3 Rs are not solely principles for humane treatment but may also enable better science.

In summary, this session provided a rare and valuable opportunity to take the concept of animal welfare into the wild, where a great many species (and individuals within species) are adversely affected by human activity, which sometimes includes scientific inquiry. While application of the 3 Rs in the context of wildlife science is still largely at an exploratory stage, we are optimistic that the continued inclusion of wildlife science-focussed sessions in future world congresses – and the new relationships built during these interactions – will considerably advance progress.

Session IV-4

Multi-Imaging Modalities, Telemetry, and the Three Rs

Co-Chairs: Patricia Turner, University of Guelph, Canada
Fraser Darling, The Institute of Cancer Research, UK

This session explored the use of new technology to refine and reduce the use of animals in fundamental and applied research through the use of radiotelementry, automated blood sampling/delivery systems, and the use of various imaging modalities combined with gene expression profiling.

Direct blood pressure monitoring by radiotelemetry was described for a mouse placental development study that proved critical for determining whether maternal changes in blood pressure with pregnancy were mediated by the fetus or NK cells of the mother. Previous attempts to monitor blood pressure using indirect measurements were unsuccessful and had led to an incorrect assumption that hemodynamic changes with pregnancy were different in mice compared with humans. These studies necessitated instrumentation of additional mice because of periodic recording failure in instrumented mice; however, the absolute numbers of mice required for these analyses was reduced by as much as 12-fold from traditional pregnancy time course studies.

The use of radiotelemetry combined with automated blood sampling systems was described for rats instrumented with probes for direct blood pressure monitoring and indwelling intravascular catheters. In addition to routine recordings of blood pressure, body temperature, electroencephalograms, and activity, modifications of the radiotelemetry probes have permitted measurement of functional renal parameters, including renal plasma flow and glomerular filtration rate. Concurrent measurement of serum corticosterone levels have suggested that instrumented animals experience less stress and physiologic alterations because they do not need to be handled for substance administration or serial blood sample collection. In addition to refining the use of these animals and collecting information on functional physiology that was not possible previously, this system has permitted serial comparisons within the same animal, reducing both study variation and experimental group sizes.

The use of micro-PET (positron emission tomography) imaging was described in rats exposed to neurotoxicants, and changes in brain activity were correlated with clinical behavioral changes obtained through conventional observational tests. MRI (magnetic resonance imaging) was conducted on these same animals, and it provided detailed information on changes in cerebral anatomy following toxicant exposure. These findings were correlated with changes in gene expression profiles in different brain regions. The use of these three combined technologies permitted much more detailed analysis of the neurotoxicity induced by various agents, reducing variation and enabling a much smaller group size (50% reduction) to be used for toxicology investigations.

These studies are representative of the new ways in which technology is being applied to investigate functional changes in animal models in response to experimental challenges, which has not been possible in the past. Because serial results can be collected from the same animal, there is a marked reduction in data variability, which has led to a significant reduction in the number of animals required for these types of studies. In addition, the use of methods to collect data or samples from animals that do not require direct animal handling has refined the use of animals by minimizing stress associated with these procedures when done manually. There are potential drawbacks to the use of these techniques that research groups should be aware of, including the significant cost for this equipment and technology, expertise required to instrument animals and interpret information, and the requirement for surgery for instrumentation and anesthesia for repeated imaging studies. Enhanced training of personnel, appropriate use of postoperative analgesics and supportive care, and use of rapid-acting or reversible anesthetic are examples of refinements that can be employed to minimize the potential for adverse effects with these new technologies.

Session IV-5

Can Pain Research Benefit Research Animals?

Co-Chairs: Alicia Karas, Tufts University, USA
Miki Kurosawa, Osaka University Medical School, Japan

Recent developments in deciphering animal behavior, including interpreting rodent facial expressions, hold promise for improving the welfare assessment of animals used in research. Pain in animals can be difficult to identify. The need for sophisticated, quantifiable scoring systems to study causes of pain and its treatment also points to the great need for practical "cage side" monitoring methods to guide our care of animals. This session explored ways in which some exciting new basic and applied research may be utilized to develop assessments and therapies particularly relevant for rats and mice.

Jeffrey Mogil from McGill University, Montreal, CA, gave the first invited lecture. A leading authority on pain testing in the laboratory mouse, he described the impasse at which pain researchers have found themselves: there is a problem with the translatability of recent advances from rodents to human clinical benefit. Drawing on research that has improved pain interpretation in human infants (facial expression), Mogil's laboratory developed and validated a well-known mouse grimace scale and, more recently, applied this technique to rats and to an attempt to refine dosing for post-surgical analgesia in laboratory mice. Thus, a technique designed to improve translatability of research from rodents to humans may also hold the key to helping us understand pain in the animals themselves.

Matt Leach, from the University of Newcastle, Newcastle upon Tyne, UK, gave the second invited lecture. His research includes developing methods for assessing and alleviating post-procedure pain and distress in small laboratory animals. He related results of his work that indicates that untrained veterinarians are no better than non-vets in assessing pain in laboratory rabbits. He also showed that training does improve reliability of assessment and that, in mice – and perhaps in rabbits – facial and body pain appearance correlate well and are promising experimental and screening tools.

Kirsten Jacobsen, University of Copenhagen, Copenhagen, Denmark, presented work from her laboratory where she studies different aspects of refining analgesia in mice and how to assess post-surgical pain in mice. Using real-time and retrospective measures, she was able to show that post-surgical pain and procedural stress can be differentiated in mice.

Shannon Duffus, the current Research Fellow at the Canadian Council on Animal Care, presented work she completed at Dalhousie University, Halifax, Nova Scotia, CA. She reviewed the problem of effective pain control in animals used as models of persistent post-surgical pain and gave preliminary support for the use of postoperative morphine in rats without a confounding impact on the research objectives.

Alicia Karas, Tufts University, North Grafton, MA, USA, introduced a potential resource for laboratory animal analgesia, modeled on a current human healthcare resource. The web-based ATLAS (Analgesic Therapy for Laboratory Animal Species) would exist as a dynamic and flexible means to inform researchers and veterinarians reviewing protocols for laboratory animals.

In summary, this well attended session informed participants of the potential for integrated strategies using basic, clinical, and back-translated evidence (i.e., from one species to another) to provide better analgesic therapy for research animals. We can conclude from this that pain research can benefit not only human welfare but also that of animals.

Session IV-6

Broadening the Application of Refinement

Co-Chairs: Hanno Würbel, University of Giessen, Germany
Kathryn Bayne, AAALAC, USA

Background
Refinements have successfully been applied to reduce noxious experiences. This session examined refinements that enhance the quality of life of laboratory animals, including positive welfare, and examined their impact on the validity of scientific outcomes.

Presentations
Good Welfare-Good Science: Refining toxicological procedures for cynomolgus macaques (Macaca fascicularis) through enhanced socialisation with care-staff by **L. Tasker**, **J. Kelly**, and **H. M. Buchanan-Smith**

Quantitative data were provided to demonstrate how Refinements to husbandry and procedures improve the quality of the scientific output. Compared to control macaques, those who had enhanced socialisation with care staff had better welfare (e.g., reduced fear responses) and increased the reliability and repeatability of cardiac parameters (heart rate, electrocardiogram waveforms, and blood pressure) recorded at baseline. The time invested by staff in socialisation of macaques was recouped through faster and better data collection. It was concluded that enhanced socialisation is both practical and feasible within busy laboratory environments, is good for staff morale, improves macaque welfare and quality of scientific output, and that such positive human-macaque relationships should be implemented as an important Refinement.

Translating regulatory compliance into better animal welfare – are we making progress? by **N. H. Franco**
This presentation described results from a systematic review conducted on papers reporting studies on murine models of tuberculosis and Huntington's disease across a ten year span. It was shown that despite a steady increase in the percentage of articles reporting some kind of regulatory compliance, there was no significant change in the proportion of studies having spontaneous death or moribund state as endpoints (being attributed maximum severity in the scale used). In fact, among those studies on murine tuberculosis reported to have been ethically approved (n=76), 50% were classified as maximum severity. Also, of all studies on Huntington's disease classified as level 4 (n=85), 79% had reported regulatory compliance of some sort. Thus, although there is progress in reports of regulatory compliance, and even some improvement in the implementation of humane endpoints and other refinement measures, such implementation is still very low, and a considerable potential for improvement remains unrealized. More importantly, there is a need to understand why in many studies, despite reports of adherence to ethical/welfare standards, or even ethical approval, these reviews and approvals do not translate in practice into better animal welfare and more humane science.

Refined blood sampling of rodents by **Lars Friis Mikkelsen**
This presentation focused on refined blood sampling, as blood sampling is one of the most common experimental procedures performed on laboratory animals for the analysis of biochemical, metabolic, toxicological and immunological parameters or the production of antibodies. Removal of blood can be stressful for laboratory animals because of the handling, restraint, anesthesia or discomfort associated with a particular technique, and hence it is in the interests of good science as well as of animal welfare that the potential stress should be kept to an absolute minimum. Further, sampling of sufficient volumes of blood in mice and rats can be challenging due to the relative small size of animals, and all sampling methods should make use of the most appropriate technique for the specific purpose and species. The presentation summarized and discussed a number of recently published papers and unpublished studies performed at Novo Nordisk A/S, Denmark or within the Danish CALAR research collaboration (www.calar.dk), with examples on the cardiovascular impact and recovery rate for different blood sampling methods in rats, quality of plasma sampled by different multiple blood sampling methods in mice, and blood quality and animal welfare implications of different blood sampling methods in certain mouse models. It was concluded that the choice of blood sampling method should be based on animal welfare and scientific considerations and be clearly stated and justified in all protocols, that certain animal models may require special attention for choice of blood sampling method, that handling and anesthesia are well known short term stressors in rodents, that animals should be habituated to handling, and that the possible choice of anesthesia should be clearly stated and justified in all protocols.

Promoting the Adoption of Alternatives by Developing Animal Welfare Leaders by **L. V. Medina**
This presentation highlighted ways for institutions to develop animal welfare leaders by creating opportunities for individuals to get involved in promoting higher standards for animal care and use as well as the adoption of alternatives. Several key factors contributing to the development of animal welfare leaders include support from senior management and a strong institutional foundation of a culture of animal welfare. Exam-

ples of animal welfare opportunities include the creation of a CARE group for technicians to promote animal welfare at a grassroots level, an enrichment committee, a corporate animal welfare committee, and enhanced training program. Innovative ideas to promote welfare include a dog adoption and play program with volunteers from across the institution. Promoting animal welfare results in many benefits to the animals and science.

Summary

The impetus for this session was to raise awareness that the common notion of the Three Rs concept in general, and that of refinement in particular, may be overly restrictive, and that the term refinement should be extended beyond minimizing noxious experiences within experimental procedures to include all aspects of animal care and management that may improve laboratory animals' quality of life. The five speakers illustrated this broadened meaning of the term refinement by covering a great diversity of aspects, including refinements of animal housing and experimental design, systematic reviewing of regulatory compliance, building an institutional culture of animal welfare, improving the human-animal relationship through specific training programs, and assessing the pros and cons of alternative methods of blood sampling. Taken together, this session nicely demonstrated that there is huge scope for improving the quality of life of laboratory animals in a multitude of previously unrecognized ways. Importantly, all speakers also provided evidence that such improvements benefit not only the animals, but also the research conducted with them, thereby representing refinements in the best of meanings of the Three Rs concept.

Session IV-7

Advancing Animal Welfare Training for Veterinarians

Patricia V. Turner[1], Bonnie Beaver[2], Kathryn Bayne[3], and Tsutomu Miki Kurosawa[4]
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VET 2011, also known as the International Year of the Veterinarian, is a year-long celebration of the 250th anniversary of the founding of the world's first veterinary school in Lyons, France in 1761. In essence, this also marks the birth of the veterinary profession, and the year has been noted in more than 400 special events in 70 countries around the world, culminating in the World Veterinary Congress in South Africa later in 2011. While offering an opportunity to celebrate the veterinary profession, VET 2011 also has provided an occasion to reflect on veterinary progress in many sectors, including animal welfare. This session evaluated animal welfare training for veterinarians, specifically in the North American and European veterinary curricula, as a newly recognized veterinary specialty in the American College of Animal Welfare. It also is part of the recommended training for laboratory animal veterinarians internationally and for veterinary students and veterinarians in Japan.

In 2008, the World Organisation for Animal Health (OIE) convened a meeting in Cairo, Egypt for veterinary educators on the topic of animal welfare. The OIE exhorted the 178 member countries to develop educational programs, particularly for veterinarians, to support progress of animal welfare initiatives globally. Both the Federation of Veterinarians of Europe (FVE), which represents 38 member countries, and the American Veterinary Medical Association (AVMA) subsequently held symposia to address this issue, recognizing that clear and consistent delivery of animal welfare within the veterinary curriculum was critical for fostering knowledge in this field. In 2010, the AVMA launched the Model Animal Welfare Curriculum Project, funded by the American Veterinary Medical Foundation. Working members include an international team of veterinary educators and animal scientists, with representation from the USDA, AAVMC, and the CVMA. The aims of this project are to identify essential animal welfare topics and time budgets for the veterinary curriculum, develop or identify materials to use for instruction, and examine methods of imparting and delivering this material to veterinary students. It is hoped that a report will be available in mid-2012.

Recognition of animal welfare as a distinct specialty for veterinarians is being pursued in North America with the development of the American College of Animal Welfare (ACAW, www.acaw.org). The 27 ACAW founding members have worked to develop a role delineation document that describes the core knowledge expected of entry level veterinarians who are applying to become ACAW Diplomates. This document will be used as the basis for developing formal post-graduate training programs for veterinarians in animal welfare, as well as for developing the certifying examination. The new College has petitioned the American Board of Veterinary Specialties for recognition as a distinct College and is provisionally recognized, pending final review by the AVMA Council on Education and Executive Board. A similar specialty college also is currently under development in Europe, the European College of Animal Welfare and Behavioural Medicine (ECAWBM).

Animal-based research and education is increasingly an international enterprise that draws significant public attention. The welfare of research animals, quality of scientific data, and institutional reputation significantly depend on assurance that veterinarians managing and overseeing research animal care are adequately trained and qualified. Yet, the knowledge and experience of veterinarians serving in this role can vary widely and globally. Education and training available to veterinarians in laboratory animal medicine ranges from specialty board certification to on-the-job exposure. Inadequate training can jeopardize animal health and welfare, as well as personnel and facility safety, and the entire institutional research enterprise. A collaborative effort has been undertaken by the OIE, the National Academies' Institute for Laboratory Animal Research (ILAR), and the International Association of Colleges of Laboratory Animal Medicine (IACLAM) to assess the need for harmonized international education and training of laboratory animal veterinarians via three focus group discussions held at international symposia in Finland, the USA, and Taiwan in 2010. In all, 27 countries were represented in discussions. The 106 participants discussed core competencies as well as means of assuring ongoing mentoring and applied training of veterinarians in this field. A publication outlining recommendations is forthcoming.

Animal welfare has come to be viewed as an important curriculum subject for veterinary colleges in Japan. This is partly in response to strong recent recommendations made by the Japanese Animal Welfare Society and the Japanese Association for Laboratory Animal Medicine, an organization largely composed of laboratory animal veterinarians. In most veterinary colleges, this subject is emphasized primarily during core instruction of laboratory animal medicine. Post-graduate specialization in laboratory animal medicine also is an option for veterinarians in Japan, and this entails further training in welfare and ethics, among other subjects.

Internationally, at the time of the 250th anniversary of veterinary medicine, there is keen interest in strengthening veterinary education and training in animal welfare, and a number of partnerships are being forged internationally to ensure that veterinarians are able to take a leadership role in this area. The harmonized goal of these projects is for veterinarians to work together with animal scientists, regulators, and others in various sectors and communities to enhance animal welfare globally.

Session IV-8

Ending severe pain and distress in animal experiments by 2025?

Co-Chairs: Coenraad Hendriksen, Netherlands Vaccine Institute and Department Animals in Science & Society, Utrecht University, Utrecht, The Netherlands
Gilly Griffin, Canadian Council on Animal Care (CCAC), Ottawa, Canada

Animals used in science are involved in a broad spectrum of animal experiments, e.g., to mimic human disease, or to test new pharmaceuticals. Some of these experiments can result in severe clinical implications for the animals such as paralysis, significant weight loss or tumor metastases, or in psychological disorders such as abnormal aggressiveness or anxiety. In some cases lethal endpoints are still used, for example in some challenge models for vaccine potency testing. Given this reality, we wanted to ask whether participants of the workshop considered it would be possible to replace severe experimental endpoints by the year 2025.

Challenges to ending severe pain and distress

Ending severe pain and distress can be viewed as a worthy goal for several reasons. Firstly, there is a greater societal concern about animal experimentation when studies involve pain and distress (Aldhous et al., 1999). This has been translated into legal instruments in an attempt to limit the severity levels of animal-based studies. As an example, the new European Council Directive 2010/63/EU states that 'Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals' (European Commission, 2010). Similarly in Canada and elsewhere national policies make minimization of pain and distress a requirement for approval of animal-based studies (CCAC, 1989). Secondly, eliminating pain and distress is also important from a scientific point of view. It is important to recognize the fact that not everyone involved in animal experimentation has a good understanding of the side effects of pain and distress; in terms of animal welfare but also in terms of good science. Too often investigators forget that pain and distress can also interfere with physiological parameters and consequently introduce unwanted bias or variability in experimental results (ILAR, 1992).

Technical limitations represent an additional challenge for moving away from protocols involving severe pain and distress. Full replacement of animal models, if ever possible, will require substantial investments and acceptance of paradigm shifts in various disciplines of biomedical research and testing. Despite some successes in non-animal test method development, progress has been slow and is extremely tedious and expensive. Although to a lesser degree, the same is true for using non- or pre-clinical endpoints instead of clinical endpoints.

A further challenge concerns the use of pain relieving strategies. Pain relieving drugs may not be used because of their potential side effects. For example, it is well known that morphine derivatives such as buprenorphine and Non-steroidal Anti-Inflammatory Drugs (NSAIDs) influence immunological parameters such as B and/or T cell responses.

Opportunities to ending severe pain and distress

By far the most important approach to reduce pain and distress is to build awareness of those individuals who bear responsibilities for those animals involved in experimentation including: investigators, animal care staff, and members of animal ethics committees. These individuals are key in the critical review of experimental design, test performance and procedures used, and should be the first to look for less severe protocols, e.g. by:

- developing non-animal models, such as *in vitro* methods;
- replacing clinical endpoints by non-clinical or pre-clinical endpoints (for example the use of protective antibody titers in vaccine potency testing instead of challenge with the virulent micro-organism to estimate protection after immunization);
- where clinical signs are inevitable, the implementation of humane endpoints as a way to limit the level and duration of pain and distress. Guidelines for applying humane endpoints have been published by a number of organizations (CCAC, 1998; OECD, 2000); and
- providing effective pain relief by ending the test procedure, administrating analgesics or providing additional supportive therapy.

Building awareness of pain and distress should stimulate consideration of the above approaches. This should be included in any curricula aimed at educating young scientists. For instance, submission requirements for a thesis where animal-based studies are reported might include the need for a section describing considerations on pain and distress and how this aspect was dealt with in the studies. Where no pain relief was given, the author should be asked to present the rationale with consideration given to how the potential consequences of pain and distress on experimental outcomes were addressed. The ethical and physiological consequences of pain and distress should also be part of training curricula of investigators and animal technicians. The role of animal ethics committees is to safe-guard the effective implementation of severity-reducing opportunities in submitted protocols, and to increase commitment to minimizing pain and distress. It is also important for animal ethics committees to develop a long-term vision on how to reduce pain and distress, and how to integrate this vision in an institutional policy on animal experimentation.

Many believe that non-animal methods are the way of the future as they provide more in-depth and mechanistic information compared to the current animal models and are generally more

economic in terms of costs and running time (NRC, 2007; De Mattia, 2011). There are also several technical developments with a potential spin-off to reducing severity levels. For instance, omics technologies are increasingly being investigated as early biomarkers, e.g., in carcinogenicity testing. Instead of studying rats lifelong for a chemically induced increase of tumor frequency, indicators for DNA damage and failing DNA repair can provide information early in life, long before DNA damage is expressed in tumor development. Another example of methods that ultimately might allow for the use of pre-clinical endpoints is the category of non-invasive and real-time technologies, such as NMR, Pet-Scan and bioluminescence. The latter technique for instance, enables the tracking of a fluorescence-labeled micro-organism (Andreu et.al., 2011) or tumor cell (Lim et al., 209) in the complex organism. Criteria for euthanasia of each individual animal can then be defined on the progression of the infection or tumor rather than on the pre-defined clinical parameters, resulting in an earlier end to the study and less pain and/or distress for the animal.

Conclusions

It was generally agreed by participants in the lunch-time workshop that additional studies to overcome the technical limitations in reducing severity are urgently needed. In the case of non-animal model development and pre-clinical endpoints this is likely to be a win-win situation as severity reduction will be the spin-off of technical developments where the primary aim is to improve the quality of science. The same might be true for development of new or optimized analgesics as effective pain reduction without side effects will increase animal welfare as well as the quality of animal data. There were also suggestions to study more refined approaches to characterize pain and distress in laboratory animals. The facial expression of mice was given as an example of such an approach (Langford et al. 2010). Additional studies require additional funding, but also additional education and training to encourage young scientists to develop an interest in these issues.

At the workshop we also asked whether it would be possible to implement a ban on animal experiments with severe pain and distress right now. The ban on the use of animals for cosmetic testing in Europe was taken as an example. Although attractive and simple, many felt that it is not a realistic approach. Disease mechanisms are complex in comparison to cosmetic testing with relatively simple endpoints such as skin irritation. Therefore developing *in vitro* models, which at the moment are relatively simple, will quite often not be possible. Many of the experiments that include severe pain and distress are for health purposes, related to debilitating and life threatening diseases such as Parkinson, arthritis or cancer. Although there is general concern in society about animal experimentation, public acceptability of studies to address human disease remains high. A side effect of a banning strategy might be that scoring systems for severity, being subjective in nature, will be disputed, and would not result in any actual minimization of pain and distress.

As a result of this workshop, we are of the opinion that increased emphasis should be placed on sensitizing those involved in experiments about the implications of pain and distress on research outcomes. We also believe that increased attention should be placed on identification of suitable pain relieving strategies.

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