Theme IV
Animal Welfare for Refinement and High Quality Science

Session IV-1: Indicators of animal welfare to implement refinement

Session IV-1: Oral presentations

IV-1-072

Affective states and the assessment of laboratory-induced animal welfare impacts

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Animal welfare is increasingly understood in terms of a wide range of affective states or feelings animals may experience. Some of these can be assessed and others, as yet, cannot. Reference is made to a comprehensive system for ranking the impacts of scientific procedures on the affective states or feelings that can be assessed. To date, the predominant focus has been on pain and distress. It is argued that, in addition to these, other negative subjective and emotional experiences including the following should be considered: thirst, hunger, nausea, breathlessness, dizziness, debility, weakness, sickness, anxiety, boredom, fear, frustration, helplessness and loneliness. Moreover, negative impacts on positive emotional states or experiences such as satiety, vitality, reward, contentment, curiosity and playfulness should also be evaluated. The purpose here is to reinforce the principle that because animals may have bad or good experiences at our hands, we have an obligation to treat them considerately (at the very least); this translates into minimising the harm we do to them and maximising the good. More effective harm minimisation should result when the Three Rs are applied to mitigating this wider range of negative emotional experiences, and when other measures are adopted that promote specific positive emotional states or the general wellbeing of animals. It is anticipated that this broader perspective will enhance caring and empathetic attitudes towards animals among investigators and members of Animal Ethics Committees or Animal Care and Use Committees.

IV-1-610

The sensitivity of animals and application of the Three Rs

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It may be impossible to fully understand the point of view of a non-human animal, yet we are duty bound to come as close as possible. To this end, we try to determine the right level of anthropomorphism, closely study their natural history, and mentally add or subtract sensory or psychological "modules" from the equivalent human experience. In practice, however, humans typically care for animals as if they sense only what we sense, and understand their existence in our terms, if at all. Even when a

species is known to respond to particular light levels and cycles, to human presence and expectations, or to stimuli outside our perceptions, this may not result in husbandry changes. Indeed, we might try to eliminate these factors, as if experiences outside our reach deserve only to be eradicated. Established abilities, such as how a pigeon returns home or a dog senses cancerous cells, were neglected for decades because we were ignorant of the mechanism and were probably unwilling to tackle such a void in our understanding. Our protocols might change considerably if we countenance the possibility that animals know their own fates, see into our minds, and have access to everything that happens around them; or if we tried seeing animals as participants in, rather than subjects of, their fate. Would we be more careful, more frugal, and more likely to seek alternatives if one of the groups that we had to sincerely explain the research to was the animal itself?

IV-1-609

Reliance on behavior as a metric of animal welfare

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A first-tier method of assessing an animal's welfare is often a judgment based on observable behavior. Several factors may confound the observation and interpretation of the behaviors observed, which could result in missed opportunities to implement the Three Rs, especially refinements. Alternatively, the refinements initiated to address a perceived problem may not correlate with the issue because of misinterpretation of the observed behaviors. Typically, research animals are observed during normal working hours while routine activities are ongoing in the animal room. Yet, the benefits and limitations to assessing animal welfare at this time have not been analyzed across species. Also, factors such as the skill level of the individual making the observations must be considered in a system of reliance on behavior to detect welfare issues. Perhaps most importantly, responses of animals to experimental procedures, their environment, etc. vary among individuals and according to species, age/maturity, gender, physiological and pathological state, environment, phase of response to a stimulus, and other factors. Thus, any tendency to extrapolate assessment criteria among individual animals, strains and species is fallible. Yet, appropriately gathered and interpreted, behavior observations can be an important and practical tool to assess welfare and validate that refinements implemented address not only extant behavioral concerns but the underlying welfare issue. A framework for implementing a pragmatic approach to using behavior as a welfare indicator will be discussed.

IV-1-069

Complementary roles for systematic analytical evaluation and qualitative whole animal profiling in welfare assessment for Three Rs applications

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Application of the Three Rs involves prospective evaluation of potential negative welfare impacts of scientific procedures, while assessment of the success of Three Rs applications requires retrospective evaluation of the actual impacts. Two complementary approaches to animal welfare assessment are available to assist with this: Systematic Analytical Evaluation (SAE) and Whole Animal Profiling (WAP). SAE aims to comprehensively anticipate functional disruptions, rank scientific procedures according to their actual negative impacts, and guide the development and application of methods to mitigate such impacts. A key focus of SAE is to assess the likely impacts on subjective mental states, adduced from objective behavioural, physiological and pathophysiological knowledge. In contrast, WAP involves observers scoring subjective impressions of appearance, demeanour and behaviour in terms of overall welfare status at the time of the evaluation. Conclusions based on qualitative WAP have been validated using key quantitative behavioural and physiological indices of welfare status. These two approaches are complementary. For example, WAP can be used to verify welfare impacts anticipated using SAE, whereas SAE may be used to elucidate the factors that contribute to a particular welfare state identified using WAP. It is suggested that combining both approaches in assessments of laboratory animal welfare will facilitate more thorough evaluation of welfare status, enable immediate or future mitigation strategies to be identified, and thereby enhance application of Three Rs measures.

IV-1-520

Behaviour changes during rat euthanasia may be a poor indicator of aversion

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Laboratory rodents are commonly euthanized with carbon dioxide (CO_2), but approach-avoidance, avoidance-avoidance, and total dwelling time studies have shown that rats find exposure to the inhalant anaesthetic isoflurane less aversive than exposure to CO_2. The aim of this study was to describe gross behavioural changes during euthanasia with CO_2 and isoflurane to determine how well these behavioural changes relate to the aversion experiments. Thirteen male Sprague-Dawley rats weighing (mean ±SD) 466 ±69 g were euthanized with CO_2 delivered at a flow rate of 23% cage volume per minute, and 13 male Sprague-Dawley rats weighing 452 ±65 g were euthanized with 4% isoflurane delivered in oxygen at 23% of the test cage volume per minute. Trials were video recorded and rat behaviour was scored for activity (number of transitions between quadrants) and rearing (two front paws off the ground) from 90 s before gas delivery began until rats ceased all purposeful movement. The frequency of each behaviour was recorded in 10 s intervals. Activity increased in both treatments after gas delivery began, with no difference in peak activity between treatments (mean ±SE; CO_2: 2.5 ±0.3 vs. isoflurane 2.7 ±0.6; t = 0.24; P = 0.81). Rats showed a higher peak frequency of rearing when exposed to isoflurane (1.2 ±0.2) than when exposed to CO_2 (0.8 ±0.3; t = 2.98; P = 0.007). Given that multiple experiments have shown that isoflurane is less aversive that CO_2, we conclude that the behavioural differences are due to an excitatory phase during induction with isoflurane. These results illustrate that observations of gross behaviour during euthanasia may be a poor indicator of aversion.

IV-1-490

Impact of simple environmental improvements on affective behavior, physiology and immune system reactivity of C57BL/6 and BALB/c mice

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The objectives of this study were to evaluate the long term effects of enrichment and housing on BALB/c and C57BL/6 mice, with particular consideration of the effects on behaviour, body weight, fecal corticoid levels, and immune system function and responsiveness (changes in complete blood cell count and response to lipopolysaccharide challenge). The study was 20 weeks in duration and involved 160 mice; 20/sex/strain/housing paradigm. Mice (5/cage) were randomized into one of two housing paradigms in solid bottom caging: contact hardwood chip bedding, or contact hardwood chip bedding + cotton nesting material + clear amber tube + 10g wood wool + 1 Cheerio 3x/week. At 20 weeks, C57BL/6 mice were heavier than BALB/c mice and enriched mice had mild but consistently greater body weights than unenriched mice. Significantly less barbering was noted in enriched female C57BL/6 mice than unenriched B6 females. Mice were videotaped monthly and behaviours were scan scored. Dominance behavior was significantly more frequent in unenriched than enriched cages. Abnormal and aggressive sexual behavior was observed in male BALB/c mice and increased over time, with significantly increased intensity in mice in unenriched cages. There were no significant housing differences for stereotypic behavior, eating, locomotion, or positive social behaviours. Feces were collected monthly and fecal corticoid metabolites were extracted and evaluated. BALB/c mice had significantly higher levels of fecal corticoids than C57BL/6 mice for both sexes. Unenriched BALB/c males had significantly lower fecal corticoid levels during the dark phase than their enriched counterparts, suggesting a blunting in Circadian corticosterone release. Within each strain and sex, there were no differences in hematologic parameters for mice housed in either caging paradigm at month 1 or 5. Further, there was no consistent effect of housing paradigm on WBC and lymphocyte subset

relative ratios in response to LPS injection, although strain- and sex-specific differences were noted. In conclusion, consistent provision of simple environmental improvements to B6 and BALB/c mice led to production of larger mice with consistent decreases in cage aggression, barbering, and abnormal sexual behaviour. Inexpensive cage improvements do not significantly alter many physiologic parameters within a particular sex or strain of mouse but may improve overall animal well-being, in particular, through a reduction in cage aggression.

Session IV-1: Poster presentations

IV-1-061

Assessment of post-surgical pain in mice using species-typical burrowing behavior

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Detection of persistent pain of a mild-to-moderate degree in laboratory mice is difficult because mice do not show unambiguous symptoms of pain or suffering using standard methods of short-term observational or clinical monitoring. This study investigated the potential use of burrowing performance – a spontaneous and highly motivated behavior – as a measure of post-operative pain in laboratory mice. The influence of minor surgery on burrowing was investigated in adult C57BL/6J mice of both genders in a modified rodent burrowing test (displacement of food pellets from a pellet-filled tube) within the animal's home cage. Almost all (98%) healthy mice burrowed (mean latency 1.3 h, SEM 0.5 h). After surgery without pain treatment, latency of burrowing was significantly prolonged (mean Δ latency 10 h). Analgesic treatment using the anti-inflammatory drug carprofen (5 mg/kg bodyweight) decreased latency of burrowing after surgery (mean Δ latency 5.5 h) to the level found in mice that had been anesthetized (mean Δ latency 5.4 h) or had received anesthesia and analgesia (mean Δ latency 4.6 h). Analgesia during surgery was associated with a significantly earlier onset of burrowing compared to surgery without pain treatment. A distinct gradation in burrowing performance was found ranging from the undisturbed pre-operative status to the intermediate level following anesthesia/analgesia and surgery with analgesia, to the pronounced prolongation of latency to burrow after surgery without pain relief. In conclusion, post-surgical impairment of general condition, probably mainly attributable to pain, can be conveniently assessed in laboratory mice on the basis of the burrowing test.

IV-1-120

Defining metrics to measure and communicate progress of 3Rs investments and activities – European Federation of Pharmaceutical Industries and Associations (EFPIA)

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In order to make a self-assessment of how its members have implemented the 3Rs, EFPIA conducted a survey with input from 18 companies, representing a range of therapeutic areas and geographical locations. One conclusion from this survey was that there were no commonly agreed key performance indicators (KPIs). Therefore, measuring and communicating the impact of 3Rs activities is very difficult. The current situation does not reflect all the 3Rs initiatives within industry. It is important to stress that the implementation of the 3Rs is a continuous effort, integrated in science as it advances.

The questions "how much do you spend?", "how many animals did you save?", and "how does it improve science?" are the most frequently asked in public and internal company debates on animal use. Establishing KPIs in relation to the implementation of the 3Rs is a challenge, as direct links to projects and the business are not visible. The total numbers of animals used in R&D-projects is not a valid KPI. This number is influenced by multiple variables such as discontinuation or launch of projects, opening or closing research labs, or changing regulatory requirements. The impact of 3Rs developments can be viewed from different perspectives (e.g. ethical, scientific, resource). Thus a single KPI covering all perspectives might be unrealistic. The EFPIA group on Research and Animal Welfare collated examples of potential KPIs to try and define a common set of indicators which could be used by its members in order to provide evidence of the benefits of 3Rs implementation.

IV-1-144

Assessment of intraplantar FCA-induced mechanical hypersensitivity using dynamic weight bearing

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Chronic pain affects one in three people over the course of their lifetime and is very poorly treated. Animal models are an integral part of pain research; however, current models tend to rely on evoked responses. There is belief that non-evoked responses may be a more relevant behavioural readout, as the animal responds in a more natural manner.

The standard incapacitance model involves placing a mouse, under light restraint, in a small (4x4 cm) Perspex chamber with each hind paw on a single pressure transducer. However, this places the mouse in an unnatural situation which may produce additional stresses in the animal. Dynamic weight bearing (DWB) is much less invasive, as the animal is placed in an enclosed area (11x11 cm) on top of a sensor containing 1936 pressure transducers and is allowed free movement. This enables assessment of the animal's natural stance during testing, which may be more clinically relevant.

Here, using the DWB test, it has been shown that intraplantar injection of Freund's Complete Adjuvant (FCA) (30 µl, 1 mg/ml) produces a significant ($P < 0.001$) reduction in weight placed through the injured hind paw for 4 days post FCA injection compared to vehicle (mineral oil, 30 µl) treated mice, which is indicative of inflammatory mechanical hypersensitivity. At 48 h post FCA injection, celecoxib (30 mg/kg p.o.) significantly ($P < 0.05$) reduced this FCA induced mechanical hypersensitivity compared to vehicle (1% methylcellulose 10 ml/kg p.o.) treated mice. This finding is in keeping with data previously acquired using the standard incapacitance model, but here only 6 mice were used per group rather than the normal 12. The DWB test is a refinement of the standard model and has the potential to reduce animal usage by half with no loss of data integrity.

IV-1-160

CO_2 and inhalent anaesthetics for the induction of euthanasia in mice: a comparative study

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The use of carbon dioxide (CO_2) for euthanasia in mice has been strongly criticised concerning animal welfare. Alternatives have not been sufficiently tested. Here, we investigate distress induced by exposure to CO_2, isoflurane and sevoflurane. NMRI mice were exposed to 100% CO_2 with different filling rates – 20% ($CO_2$220), 60% ($CO_2$60), and 100% ($CO_2$100) of chamber volume/min – or isoflurane and sevoflurane in different concentrations (Iso2%, Iso5%, Sevo4.8%, Sevo8%). We recorded behaviour and vocalisations during induction until surgical tolerance (ST) or during 5 min of air exposure (control). Then, mice were decapitated and glucose, adrenaline and noradrenaline were measured.

ST was reached fastest after exposure to $CO_2$100, followed by $CO_2$60 < Iso5% < Sevo8%. 37.5% of the mice did not reach ST within 5 min while exposed to Iso2% and Sevo4.8%. With $CO_2$20, 75% of the mice did not reach ST. Compared to control, changes in behaviour were apparent regarding grooming, arousal, escape behaviour and excitatory phenomena. No audible or ultrasound vocalisations were detected. Glucose concentrations had risen in Iso2%, Iso5%, and Sevo4.8% groups compared to control. Adrenaline and noradrenaline concentrations were increased in $CO_2$60 and $CO_2$100 treated mice compared to all groups.

Even though $CO_2$60 and $CO_2$100 induce narcosis faster than isoflurane and sevoflurane, the increases of adrenaline and noradrenaline point towards a higher perception of distress. Further investigations (histopathology of respiratory tract) are in progress to conclusively determine if isoflurane and sevoflurane in higher concentrations can be recommended for the induction of euthanasia in mice.

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IV-1-236

The effect of transportation on the physiology and behaviour of rats

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Transportation of laboratory rodents unavoidably causes stress. Nevertheless, very little is known about the effects of transportation and how long it takes for the animal to recuperate. To obtain reliable scientific results from experiments using animals, their physiological status needs to be normalized / stabilized to a condition which can be defined as baseline. Using stressed animals is likely to result in considerable and unintended effects on research results. We investigated physiological and behavioral parameters before and after transportation, as well as in transported and non-transported animals. Blood samples were taken for analysis on plasma corticosterone, glucose and creatine kinase. Physiological measurements were performed by means of telemetry, measuring heart rate, blood pressure and activity. Behavior was measured by means of home cage observations. Besides measuring these parameters, a study was dedicated to the effect of temperature fluctuations during transportation on the core body temperature of rats.

Temperature inside transportation boxes strongly correlated with body temperature. Significantly decreased body weight, and water and food intake were observed on the day of transportation in transported animals. Plasma corticosterone levels were increased up to at least 16 days after transportation. Female control rats showed decreased glucose levels compared to transported females on the day of transportation. Blood pressure and heart rate showed a lasting decrease after transportation. Grooming increased, while social interactions and locomotor activity decreased after transportation. With these studies, we have demonstrated that there is a long lasting effect of transportation on physiological and behavioural parameters.

IV-1-270

An interactive tool used to improve early recognition of health problems in mice

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Early recognition of a health problem allows prompt reporting, fast intervention and appropriate management of an animal in pain or in distress. To facilitate early detection of health problems, we built an extensive collection of photographs and videos illustrating physical and behavioral clinical signs as well as abnormal phenotypes observed in our experimental mice and mouse colonies. We used a screenshot software to highlight and annotate the photographs which allows quick recognition of the condition. In addition, several photographs of the same condition show the progression of clinical signs. This extensive collection (over 250 photographs and videos) is available through our internal network for consultation by the veterinary staff, animal care technicians and husbandry personnel. Specific pictures are posted on animal room doors to alert the animal care personnel of a possible health or welfare problem concerning a specific strain of mice. The collection is used as a teaching tool for our staff, investigators, interns, students and members from external institutions. We are developing an interactive webpage on our internal network, where it will be possible to search for pictures, appropriate terminology, explanations and references, using a medical term or an animal model. We observed that trained individuals increased the number of cases reported and improved their signalling of health problems which allowed early evaluation of the animals by the veterinary staff. This interactive tool has become a key element in improving the animal welfare for the mice housed in our institution.

IV-1-292

Eliminating pain and distress in ocular safety testing: use of topical anesthetics, systemic analgesics, and humane endpoints

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Current EPA and OECD test guidelines for the rabbit eye test only allow the use of topical anesthetics when the user demonstrates that such pretreatments do not interfere with the test results. This requirement often results in topical anesthetics and other pain relieving medications not being used. ICCVAM subsequently evaluated the potential impact of using topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress when the rabbit eye test is required for ocular safety testing. ICCVAM concluded that balanced preemptive pain management should always be provided when the rabbit eye test is conducted for regulatory safety testing. This should include pre-treatment with a topical anesthetic and a systemic analgesic, followed by post-treatment with systemic analgesia until lesions resolve or the study is terminated. ICCVAM recommended regular monitoring and recording of all clinical signs that may be indicative of pain and/or distress, as well as recording of the nature, severity, and progression of all eye injuries. ICCVAM also recommended several additional types of ocular damage that should be used as humane endpoints to end studies earlier. US agencies have endorsed these ICCVAM recommendations, which will effectively eliminate pain and distress in most *in vivo* ocular safety testing situations, thereby significantly refining animal use. These refinements should be routinely used whenever the rabbit eye test is still required. A proposal to revise OECD TG 405 with these modifications is currently under consideration.

IV-1-296

Assessment of the effects of meloxicam on polyclonal antibody production and related adjuvant-induced inflammation in New Zealand White rabbits

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Complete and Incomplete Freund's Adjuvant (CFA/IFA) are often used in the production of antibodies to specific molecules and can cause local inflammation and granulomatous reactions which can be painful if abscess formation or skin ulceration occurs. Treatment of significant skin lesions may include the use of analgesics. We investigated whether the non-steroidal anti-inflammatory drug (NSAID) meloxicam, dosed for analgesia, would impact antibody production. We hypothesized that meloxicam treatment would not impact on antibody response. Thirty New Zealand White (NZW) rabbits received immunization with adjuvant over 7 months (1 CFA inoculation/3 IFA boosts). Half of the rabbits were randomly selected to receive meloxicam (0.75 mg/kg BID for 3 days, followed by 0.3 mg/kg BID for 7 days) in piña-colada flavored tablets, while the remaining received placebo tablets. This dose of meloxicam was well-tolerated with no clinical indications of gastrointestinal ulceration or nephrotoxicity. There was no significant difference between groups in food consumption, body weight, clinical pathology parameters, and number of nodules or ulcerated lesions. There was also no significant effect of treatment (n = 15/treatment group) on antibody titer levels determined by linear regression with an optical density target of 0.5 [$t(28) = 0.1579$, $p = 0.8757$], optical density target of 0.1 [$t(28) = 0.5031$, $p = 0.6189$], or evaluation by 3 times the background reading [$t(28) = 0.3560$, $p = 0.7245$]. Plasma IFN-gamma and IL-12, two cytokines associated with Th1- and Th-2-type immune responses, were evaluated by rabbit-specific ELISA. In summary, analgesic doses of the anti-inflammatory drug meloxicam do not alter antibody production in a traditional immunization protocol for polyclonal antibody production.

IV-1-309

Refinements in dog housing and husbandry, and the link with quality of science

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Dogs have special protection under the UK legislation on the protection of animals used in scientific research. It is critical that their welfare is maximized, and that the most reliable and valid scientific results are achieved from their use. Whilst the link between good welfare and good science is often made, housing and husbandry practices are often advocated without a sound scientific understanding of their welfare implications. In collaboration with academia and industry, we are developing a project to examine the link between Refinements in dog rearing, housing and husbandry and quality of scientific output, measured in terms of repeatability of data, and between-dog variability. AstraZeneca has recently built new facilities and has incorporated many design features aimed to improve dog welfare. These include pens with raised platforms, glass panels to improve visibility and reduce noise from barking and access to both indoor and outdoor runs with a variety of structural enrichments. In this presentation, we describe Refinements in housing and husbandry in the new facility and present preliminary data on physical, behavioural and cardiovascular measures on telemetered dogs. We describe our plans to incorporate further Refinements throughout the life of the dogs, especially in enhanced socialisation with humans, habituation to procedures and positive reinforcement training, and how these impact on quality of scientific data output.

IV-1-360

The effect of behavioural state and cage environment on responses to euthanasia with isoflurane or carbon dioxide in BALB/c mice

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Two common euthanasia agents, isoflurane and carbon dioxide (CO_2) cause aversion and stress. In addition, "procedural" stressors (handling, placement in an unfamiliar environment, etc.) may add to the overall stressfulness of euthanasia. To investigate this we carried out euthanasia in BALB/c mice (24 males and 24 females, 8 mice/group) with isoflurane (5% isoflurane in 20% cage volume/min oxygen) or CO_2 (20% cage volume/min) using three protocols:

A. euthanasia in empty cages into which mice were placed immediately prior to euthanasia, analogous to normal procedure;
B. home-cage euthanasia whilst initially sleeping; mice were acclimatised to the cage for ≥24hr and provided with bedding and nesting material;
C. home-cage euthanasia as above, but carried out during wakefulness.

Gas flow initiation caused sleeping mice to awaken in 13.5 ±1.3 s for Isoflurane and 10.1 ±1.2 s for CO_2 (difference not significant, $P = 0.077$). Time exposed to a euthanasia agent whilst awake was significantly shorter for mice exposed to Isoflurane (56.3 ±2.8 s) during sleep than for CO_2 (86 ±4.2 s) or animals which were awake when exposed to Isoflurane (77.4 ±3.6 s) ($P < 0.001$). Mice exposed to isoflurane showed significant increases in behaviours potentially associated with excitation and stress such as running and ataxia. Running occurred only during isoflurane exposure and more frequently amongst un-acclimatised mice than mice exposed in home cages ($P = 0.01$).

In conclusion, isoflurane appears to be less alerting than CO_2, taking longer to cause awakening. Initiation of euthanasia during sleep can significantly reduce the amount of time to which animals are exposed to euthanasia agents during wakefulness, and hence potentially reduce the time animals are stressed. Home-cage euthanasia reduces some signs of agitation or excitation.

IV-1-393

Environmental enrichment influences the results in behavioral tests

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An enriched environment is suggested to increase the welfare of captive animals. We tested whether such an environment can influence the performance of laboratory rats in tests commonly used in behavioral neuroscience. Eleven adult rats were individually housed in standard shoeboxes with litter and food/water ad lib; 11 other rats were housed in shoeboxes each containing hardwood blocks, Kraft paper towels and a non-toxic PVC tunnel. Rats were tested after 12 weeks. Compared to the non-enriched condition, the enriched environment showed no effects on the Sucrose Preference Test, Emergence Test, Open Field and Elevated Plus Maze. However, the enriched environment was associated with decreased swimming time in the Forced Swimming Test and decreased exploration time in the Novel Object Test. These data indicate that environmental enrichment can influence the baseline of laboratory rats on behavioral tests aimed at cognitive performance.

IV-1-461

Welfare assessment in swine in biomedical research – suggestion for a welfare assessment standard for research facilities

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The swine (*Sus scrofa*) is becoming an increasingly attractive experimental animal species as an alternative to rodents and non-human primates. Despite the fact that a considerable amount of biomedical research has been done on swine, hardly any studies include systematic welfare assessment of laboratory swine. In order to quantify and control laboratory swine welfare, a practical tool is needed. The purpose of this presentation is to suggest a welfare assessment standard for research facilities, primarily based on an exposition of ethological considerations relevant for the welfare of swine in biomedical research. The tools for porcine welfare assessment presented suggest a method for monitoring the welfare status of individual laboratory swine, which is intended to improve practical scoring of the welfare of individual swine, the interpretation of the findings, as well as communication between researcher and animal caretakers.

IV-1-466

The TIN score: assessment tool for distress in laboratory mice

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The ability to assess well-being in laboratory mice is crucial to maintaining quality research. If mice are distressed or in pain, protocols can be adjusted and humane endpoints can be determined more accurately. Lack of well-being might be assessed by observing decreases in natural behaviors. We previously observed that a new piece of nesting material, dropped into a cage of healthy mice, will be incorporated into the nest within 10 minutes – a normal response we called a TIN score (= Time to Incorporate Nest material). If mice completed this task within 10 minutes it was considered a positive TIN score. We observed mice before and after undergoing procedures ranging from mild to severe and determined their TIN score. We found that mice undergoing mild procedures such as osmotic pump placement and ovariectomy showed no change in their TIN score one day after the procedure. However, mice took much longer to regain their baseline TIN score if they underwent more severe procedures such as carotid injury surgery. Differences in TIN score also identified a difference in recovery rates of single versus group housing conditions in the carotid injury group. Additionally, we found we were able to discern differences in diabetic mice that were sick prior to surgical procedures. In summary, our preliminary evidence suggests that the TIN score is a potentially useful tool for assessing well-being in laboratory mice and further testing should be done on this method in order to expand the clinical significance of its application.

IV-1-467

Environmental enrichment for NTP studies

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Environmental enrichment has been described as any measure that promotes expression of species-specific natural behavior and inhibits abnormal behaviors. Enrichment is beneficial for the psychological and physical well-being of animals. Recent modifications to the National Toxicology Program (NTP) animal care and use program represent an important effort to include environmental enrichment in NTP rodent studies. These modifications fulfill the Guide for the Care and Use of Laboratory Animals (Guide) and AAALAC International requirements, enhance animal well-being by providing sensory and

motor stimulation, improve quality of experimental data, and allow animals to have choices and control over their environment. Within the framework of NTP study requirements, social and physical enrichment were considered appropriate enrichment options. Within this context, social enrichment of group housing allows animals to perform social behaviors such as grooming, vocalization, and play. The social nature of rodents readily allows for successful group housing of male and female rats and female mice. However, to offset behavioral issues in male mice, group housing requires introduction of mice in stable environments at weaning. Physical enrichment devices allow animals to control the stressors in their environment by enabling species appropriate behavior, e.g., nesting for mice, gnawing or burrowing and perching for rats. As the NTP moves toward instituting an enrichment program for future studies, several factors are being considered that will address the need to provide enrichment for animals on study without compromising the scientific question(s) under study.

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Rat aversion to isoflurane and carbon dioxide

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Laboratory rats are commonly euthanized with carbon dioxide (CO_2). The inhalant anaesthetic isoflurane appears to be less aversive than CO_2, but little research has directly compared rat aversion to the two gases. We used an aversion/avoidance experiment to compare aversion to isoflurane and CO_2. Albino Sprague-Dawley rats were given the choice between staying in a dark compartment filling with CO_2 (n = 8) or isoflurane (n = 8), or escaping to a compartment with a light intensity that rats find slightly or highly aversive (300 or 1600 lux). The flow rates of each gas, respectively, were the ones recommended for euthanasia to mimic real euthanasia. When tested at the high light level, none of the 8 rats left the dark compartment filling with isoflurane, but 6 of 8 rats left the dark compartment filling with CO_2 (Fisher-Exact test, P=0.004), indicating that aversion to CO_2 is higher than aversion to isoflurane. At the lower light level, 6 of 8 rats left the dark compartment when exposed to the isoflurane, and 8 of 8 left when exposed to CO_2 (N.S.), suggesting that isoflurane is moderately aversive. When rats were re-exposed to the two gases, all left the dark compartment, regardless of light level. However, rats remained longer when exposed to isoflurane (23 s ±3 s) versus CO_2 (15 s ±3 s; $F1,14 = 5.46$, $P < 0.035$). Together these results indicate that isoflurane is less aversive than carbon dioxide to laboratory rats, and thus preferable for use in euthanasia.

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Use of thermography as refinement indicator of animal welfare

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Thermography is the technique of measuring natural thermal radiation from body tissues of clinical interest. It is safe, requires no control of environmental conditions, and is non-invasive. The technique has been used for different applications, among others to study inflammatory processes in joints, and skin temperature in connection to pain or pruritus. This paper describes different application areas of thermography in laboratory animal protocols, and focuses on the use of digital infrared thermal imaging in a rat collagen induced arthritis model as a method for evaluating disease progression and pain. The presentation gives other examples of the use of thermography in laboratory animal science contributing to welfare evaluation of the animals.