



Session IV-6: Broadening the application of Refinement

Session IV-6: Oral presentations

IV-6-078

Refined blood sampling of rodents

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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.

Sampling of sufficient volumes of blood in mice and rats can be challenging due to the relatively small size of animals. All sampling methods should be evaluated and the use of the most appropriate technique for the specific purpose and species or subspecies is important. Sampling by trained and competent staff is essential for ensuring that any pain, distress or discomfort is kept to a minimum. Minimisation of such adverse effects is further important for scientific as well as ethical and legal reasons, since they can cause biological changes which may affect the blood sample, and hence the validity of the research

results and the number of animals used to achieve the scientific objective.

This presentation will summarize and discuss a number of recent published papers and unpublished studies performed at Novo Nordisk or within the Danish CALAR research collaboration (www.calar.dk) combined with relevant references in order to evaluate quality of plasma sampled by different methods, e.g. amputation of the tail tip, lateral tail incision, puncture of the tail tip and periorbital puncture, for multiple blood sampling. Animal welfare implications, advantages and disadvantages, based on scientific analysis, telemetric recordings and intense observations, by using methods like submandibular blood sampling in standard and genetically modified mice and sublingual blood sampling in rats will further be presented and discussed.



IV-6-126

Good welfare – good science: Refining toxicological procedures for cynomolgus macaques (*Macaca fascicularis*) through enhanced socialisation with care-staff

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Cynomolgus macaques (*Macaca fascicularis*) are the most commonly used nonhuman primates for research and testing in Europe. They are an important in vivo model in regulatory toxicology for predicting the adverse effects of novel pharmaceuticals on clinical pathology, cardiovascular and organ parameters. Common husbandry practices, traditional capture, handling and restraint techniques, and close proximity of human care-staff may result in physiological stress responses which confound toxicological measurements. Habituating and socialising primates to human care-staff will help to avoid or reduce fear responses and facilitate handling for routine husbandry and scientific procedures, leading to improvements in scientific outcomes.

Our aim was to determine the effects of a five-week enhanced socialisation programme with animal care staff on newly ac-

quired cynomolgus macaques in a laboratory. We used a multi-dimensional welfare assessment tool we have previously developed for cynomolgus macaques to compare a control (N=40) and matched group (N=40) of male and female juvenile macaques subject to a socialisation programme. As well as enhanced welfare we also found positive effects on cardiac parameters (heart rate, electrocardiogram waveforms and blood pressure) recorded at baseline by non-invasive digital electrocardiogram (ECG) and indirect high definition oscillometry blood pressure as part of the core battery of measures incorporated into regulatory toxicology. Our results provide support for the importance of positive human-macaque relationships for both primate welfare and quality of science.

IV-6-404

Ways to advance laboratory animal welfare not only when it is a regulatory mandate but whenever we recognize opportunities to enhance husbandry and/or research practices

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When William Russell and Rex Burch proposed the 3Rs (Replacement, Reduction and Refinement), they were suggesting this approach for all situations when animals are used for research purposes. Animal welfare and the 3Rs is an important focus for every good laboratory animal research program. Ethics committees must review animal welfare questions as part of a protocol review process and it is a highlight during training, semiannual facility inspections, and post-approval monitoring. The veterinary staff, animal caretakers and research staff are charged with ensuring high standards for animal welfare while carrying out their daily responsibilities for health care support, animal observations and/or research. Animal welfare concerns must be reported promptly to the veterinary and ethics committees for follow-up. But promoting animal welfare through the adoption of alternatives can sometimes fall short without

enough manpower or energy directed towards the 3Rs. Abbott is building a culture of animal welfare by creating opportunities for people to develop into animal welfare leaders. Examples include Abbott CARE (Caring for Animals in the Research Environment), an Alternatives Committee, an Enrichment Committee, a Corporate Animal Welfare Committee, an animal welfare award program, a 3Rs coordinator/scientist position, and a Dog Socialization and Adoption Program. Enhancing animal welfare builds employee morale, improves institutional reputation, addresses public concerns and often minimizes variability that confounds scientific investigation. This presentation will give a broad overview of ways to promote best practices for animal welfare through the development of animal welfare leaders in a local or global program.



IV-6-418

Translating regulatory compliance into better animal welfare – are we making progress?

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The 3Rs is increasingly acknowledged as a guiding principle also in legal documents such as the recent European Directive 2010/63. To assess how animal welfare and refinement have been considered in biomedical research, and how they relate to regulatory compliance, we reviewed published research over a 10-year period in two biomedical fields in which animal research is central: experimental infection and hereditary neurodegenerative diseases. We performed a systematic review of papers on murine models of tuberculosis (TB) (1997-2007, N=244) and Huntington's disease (HD) (1999-2009, N=233). A 4-level severity scale was devised for each case, based on disease stage (from asymptomatic to moribund stage / spontaneous death), invasiveness of experimental procedures and any refinement applied. Overall,

reports of regulatory compliance increased significantly ($p < 0,01$ for TB, $p < 0,01$ for HD) across the ten-year period analysed. Moreover, we found a significant ($p < 0,05$) relationship between regulatory compliance and application of humane endpoints in lethal TB studies. However, the proportion of the most severe studies did not change significantly (43% in TB, 36% in HD, overall) over time. Of these most severe studies, 49% of the TB studies and 79% of the HD studies reported ethical approval. The application of humane endpoints and other refinement measures increased, but in a considerable proportion of studies animals were allowed to reach severe levels of distress. This may be scientifically required or may be the result of insufficient refinement, as will be further discussed in the presentation.

Session IV-6: Poster presentations

IV-6-042

The appropriateness of the use of therapeutic drugs on regulatory toxicology studies and ethical considerations

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Historically there has been reticence to administer therapeutic drugs to animals on toxicology studies for fear of adversely affecting the outcome of the study. However, there are occasions where drugs to alleviate pain, inflammation or to treat general symptoms of disease should be administered as study animals should be alert, active and healthy. All logistics behind animal health ensures that only animals in good health are allocated to study. It is also clear that during a toxicology study incidents occur, such as damage to extremities, lesions and general ill health, not related to test article toxicity that require intervention.

Our objective is to explore the issues surrounding the humane treatment of animals to ensure that they are treated with appropriate therapies when the unforeseen occurs. These include the necessity of not administering drugs needlessly, trying the least invasive methods first and appropriately researching the likelihood of drug interactions with the test article.

The clinical veterinarian (CV), technical staff, study director and sponsor play pivotal roles in this process. There must be interaction between each of the members of the group to ensure appropriate actions are taken when the need arises.

The CV's inherent responsibility includes monitoring and providing recommendations concerning the following: preoperative procedures, surgical techniques, qualification of institutional staff performing surgery and providing postoperative care. Examples of classical vs. conservative treatments are listed together with the logistics of the process and the information flow between scientific personal and the CV.

The cornerstone of the whole processes is to ensure reliable data from the studies whilst respecting animal welfare.



IV-6-156

Automated blood sampling – refinement of repeated blood sampling techniques in permanently catheterized mice

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Refinement of blood sampling techniques in laboratory rodents is important to improve animal welfare and reduce variation in the experimental results. Manual blood sampling is known to be associated with a stress response, which besides being a serious welfare concern is a well-known source of experimental bias. The Accusampler® (Dilab, Lund, Sweden) can sample blood automatically, theoretically without disturbing the animal at the time of withdrawal. Furthermore, samples of 5-50 μ l can be collected, hence more samples can be taken per animal and fewer animals would be required in a study.

Automated blood sampling concurs with two of the 3Rs by potentially reducing the number of animals used when sampling blood and refining the method of repeated blood sampling. The method has been thoroughly investigated in rats. However, it is

currently unknown to what extent automated blood sampling causes stress in mice.

In a recent study, male BALB/c mice were subjected to micro surgery with catheterization of the common carotid artery and connected to the Accusampler®. Corticosterone levels in blood and feces were quantified to evaluate the stress response associated with this method. Based on both results and technical difficulties it is concluded that the method is stressful to some extent, but the technique has the potential to refine the process of repeated blood sampling.

This presentation will address technical difficulties that are associated with the method, both regarding animal welfare issues and data collection, and how these difficulties are best resolved.

IV-6-251

Group housing of male mice in long term toxicity studies

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Due to their naturally aggressive behavior, male mice are often housed individually in long-term toxicity studies, and group housing of male mice has often been described as a stressful condition. However, several studies advocate group housing of mice to enable normal social behavior and interactions between the animals. A handling procedure that makes group housing of male CD1 mice possible in long-term toxicity studies has been developed at Safety Assessment within AstraZeneca. The key factors of the procedure include allocation into groups before sexual maturation (4-5 weeks of age; 2-4 animals/cage) and thereafter a one week acclimatization period. At cage cleaning, used nesting material should be transferred from the used to the clean cages. External changes should be avoided as far as possible, e.g. the cages should be kept at the same place in the room

throughout the study. Observations on the effect on aggression/ fighting in group-housed male mice following different procedures performed in toxicity studies have shown that temporary removal of animals from the group for blood or urine sampling does not affect the group dynamics. However, temporary removal of animals for mating leads to fighting if the animals are taken back to the original group. In addition, treatment with test compound might affect the general condition of the animals and the social hierarchy could be changed. In such cases, aggression/ fighting might occur and the animals have to be separated. Our experience clearly indicates that group housing of male mice in long-term studies leads to less stressed and more easily handled animals, as compared to individually housed mice.



IV-6-312

Novel canine housing in the United Kingdom – a welfare perspective

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AstraZeneca's original dog facility in the UK was built in the 1970s, prior to the introduction of the Animals (Scientific Procedures) Act 1986. Despite alterations in the 1990s, it became clear that the facility was not fit for purpose considering Home Office recommendations and new thinking around animal husbandry and best practice.

A decision was made by AstraZeneca in 2004 to design a new dog facility for regulatory studies to incorporate the latest thinking in animal welfare and science, engineering and Health and Safety. The layout of a 36-pen unit was changed from a corridor effect to an open plan design. Individual pen size was increased

from 2.1 m² to 4.5 m². Pen dividers were manufactured from 10 mm glass to enable dogs to sit on benches within their home pen and see across an entire room. Specific indoor and outdoor exercise areas were included. Natural light was a significant feature of the new building. From a scientific perspective, the physical building and working practices were specifically designed with Good Laboratory Practice requirements in mind.

Since opening in 2008, a consistent observation has been that the dogs have been significantly quieter and easier to handle in their new surroundings in comparison to the old facility. This may be due to specific design features and working practices.

IV-6-349

Combination of a rapid screening PK method and lateral marginal vein sampling technique to generate pharmacokinetic and pharmacodynamic data from *M. tuberculosis* infected mice

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Mice are commonly used disease models for tuberculosis. Obtaining PK data from the TB infected animals provides tremendous value in terms of PK/PD analysis. However, collection of multiple blood samples for pharmacokinetics studies from TB infected animals in a Bio-safety level 3 laboratory (BSL3) environment remains a major challenge. Hence, we used a combination of a lateral marginal vein blood sampling technique with a rapid screening PK method and sampling lungs for bacterial burden to obtain both PK and PD data from the same infected mouse. Animals were sampled after multiple days of dosing to

obtain PK profiles and subsequently lungs were collected for bacterial enumeration. The PK parameters obtained from the improved technique and from the conventional method were highly comparable. The single mouse PK from TB infected animals allowed not only high quality PKPD data but also reduced animal usage by almost nine to tenfold. Thus, this rapid screening method achieved significant 3R (Replace, Reduce, and Refinement) benefit.



IV-6-370

Environmental enrichment in laboratory mice: its effects upon reproductive physiology

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Although a growing body of evidence indicates that environmental enrichment (EE) facilitates normal development and behaviour in laboratory mice, few studies have been conducted to demonstrate its impact upon male and female reproductive physiology. Our study investigated the effects of EE on the development and reproductive success of Albino Swiss mice, using PVC tubular devices and shredded paper as physical enrichment.

Animals were allocated in regular housing cages in groups of five individuals and treated as non-enriched (control, C) or enriched from weaning to adulthood (E). To evaluate the EE effects upon pups' development, a third group of females were enriched from the time the male was introduced to achieve pregnancy (EP). In males, evaluated parameters were body, testicular and accessory glands weight, sperm functional activity (motility, viability, acrosome and membrane integrity), testosterone concentration, *in vivo* fertilization rates, litter size, and embryo degeneration percentages. In females, evaluated parameters included body, uterine and ovary weight, spontaneous ovulation,

estradiol concentration, *in vivo* fertilization rates, litter size, pups neurobiological (cliff avoidance, negative geotaxis, surface righting reflex), physical (body weight at d 1, 7, 14 and 21, bilateral pinna detachment, low incisor eruption and eyes opening) and reproductive development (testicular descent, balanopreputial opening and vaginal opening).

No differences were evident in any of the parameters reflecting the basic reproductive physiology of males or females. However, a significantly higher number of pups were born from enriched mothers (C: 9.5 ± 0.6 , $n=4$; E: 10.7 ± 0.2 , $n=5$; EP: 12.2 ± 0.7 , $n=5$; $p=0.03$). A strong tendency was detected towards a faster development of the physical and reproductive parameters of pups born from both enriched groups, yet significant differences were only observed for testicular descent (day 19, C: $0 \pm 0\%$, $n=16$; E: $62.5 \pm 12.5\%$, $n=17$; EP: $21.6 \pm 9.7\%$, $n=21$; $p=0.002$). Although EE showed limited effects on reproductive physiology in this strain of laboratory mice, a faster pup development appears to be favored by an increased physical complexity in the environment from birth.

IV-6-512

Double decker caging for ferrets

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There are no USDA or NIH Guide housing space requirements specifically mandated for ferrets beyond allowing for "normal postural adjustments." Ferrets are usually housed in cage types such as large tubs or modified rabbit cages, which cannot be used in our infectious disease research work. We identified a new caging type, initially designed for rats, and worked with the vendor to adapt it to provide an enhanced ABSL2 compatible housing environment for ferrets.

In 2008, we noticed a new caging prototype at a vendor show which had been developed to allow rats to have greater cage complexity while also held in an Internally Ventilated Caging (IVC) system. This caging system was called the "Double Decker" and contained a solid shelf that fit half the cage width.

We had the idea that this caging might be just the thing we needed for our ferrets – IVC caging that was double the normal height and with the added benefit of also providing more complexity (the deck) and the vertical space for the ferrets to stand completely upright.

We tested a sample cage. The ferrets were unable to use the shelf since the solid plastic was too slippery for them to "get a grip" on and pull themselves onto effectively. However, the height was perfect! The other issue was feeder development and placement within the cage. We worked with the vendor to find solutions for both issues and have been successfully using this caging in our program now for over a year.



IV-6-709

Primate life in two American laboratories

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We reviewed long-term daily records of 146 rhesus macaques and 37 chacma baboons from two American research facilities. The study aim was to obtain a longitudinal perspective of laboratory life for primates, and to assess potential problems regarding events that cause pain and/or distress and which affect psychological well-being. We systematically tabulated data under the following major headings: demographic, housing, experiments, manipulations, chemical agents, illness and injury. On average, macaques survived 7 years in the lab, and baboons survived nearly 9 years; most were killed as part of or died during an experiment (55% macaques, 61% baboons). On average, macaques were moved 22 times, spent slightly over half their lives (53%) caged alone, and were handled (e.g., for blood collection, injection, physical exam, or surgery) every three days.

Baboons were moved 31 times, spent 41% of their lives caged alone, and were handled nearly once per week. Forty-one different parasites and microbes infected the macaque population, and nearly half suffered chronic diarrhea. Twenty macaques suffered at least one amputation (usually fingers) or avulsion, most of which were self-inflicted. Post-surgical pain relief given to macaques (0-2 days) contrasted sharply with what is provided to human patients following similar procedures (1-3 weeks). Environmental enrichment records covered, on average, 30% of a macaque's time in the lab, and 31% of a baboon's time. We discuss these and other findings in the context of legislation designed to minimize pain, distress and suffering in laboratory primates.

IV-6-719

The use of mouse inhalation insert's: in house study to investigate welfare improvements to mice while restrained for inhalation studies

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Due to unforeseen complications regarding the use of mouse restraining tubes in a large inhalation study, it was discovered that when mice were placed into restraining tubes the metal bars situated at the front section of the nose cone caused skin abrasions around the snouts of a percentage of the mice. To avoid any possible issues around the possible completion of the study due to animal welfare, an alternative solution was investigated.

The aim of this study is to produce a working solution to combat the damage caused by the current restraining tubes used in

mouse inhalation studies. This study was run in 2 parts. Firstly approximately 50 inserts were produced and used to see if moving an animal from bar tubes to an insert tube will reduce any damage caused. Secondly, providing an entire study with inserts to see if they are a good replacement for the bar tubes. If this study is successful then all mouse inhalation studies will use the new restraint tubes and inserts.



IV-6-720

Covance Animal Environmental Enrichment Program

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At Covance we ensure the well-being and needs of the animals in our care as a priority. Over the years we have tried to provide the most up to date and naturally beneficial enrichment all animals we work with to help mimic their natural environment. We currently source items and products from companies that are experienced in this field and work closely with them to produce new products that have the needs of the animals at the front of

their thoughts. As time goes on, new and more advanced products are introduced into the market which look like the next phase of improving the environment of the animals, but is it? At Covance we have a system in place that takes new items and puts them to the test. This poster shows some of the items we currently use and the process we follow.

IV-6-721

Comparative analysis of blood sampling techniques in the rat

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Blood sampling is one of the most common procedures performed on laboratory animals as part of scientific research. As an on-going commitment to quality and welfare, Covance (Harrogate), identified a need to improve the current blood sampling method used for rats.

The aim of the study was to investigate and evaluate sampling from the jugular vein (JV) as a new blood sampling technique at Covance and compare it to alternative methods, including the sublingual vein (SV), the orbital sinus vein (OS) and the lateral caudal vein (LCV), being the preferred method used at Covance.

A 22-day study was conducted comparing the LCV, JV, SV and OS as sites of blood sampling. Blood samples were taken from animals on several occasions and a number of parameters evaluated throughout the study.

The most pertinent findings noted included localised tissue damage of the tail, increased food consumption, body weight

and water consumption in animals sampled from the LCV. Animals sampled from the OS had lens opacities after blood sampling. The JV route was the only route that produced no clotted EDTA or trisodium citrate samples in week 4. When compared to alternative blood sampling methods on welfare grounds, sampling from the JV does not require the use of anaesthesia or a warming device. A major benefit of this is that blood can be taken within one minute of the animal being dosed due to the manual restraint method used, consequently reducing the stress which could potentially affect the physiological state of animals and measured blood parameters.

In conclusion, the comparative study indicated that from an animal welfare and sample quality perspective, the JV was the preferred site of blood sampling for rats. In addition, the implementation of JV blood sampling method has successfully focused on the 3R's, specifically on reduction and refinement.