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## Session IV-4: Multi-imaging modalities, telemetry and the Three Rs

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### Session IV-4: Oral presentations

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IV-4-699

## Application of radiotelemetric recording to study mouse models of gestational pathology

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Mice are valuable tools providing understanding of the physiology and pathologies of human pregnancy. Acute onset hypertension with kidney failure is the commonest (3-7%) human pregnancy complication, characterizing the syndrome pre-eclampsia, a medical emergency with immunological complications. We implanted PA-C10 radiotransmitters (DSI) into 8 week female mice. After 10 days recovery, instrumented mice were paired for mating. Upon copulation plug detection, data were collected continuously to 48 h postpartum. A fluctuating pattern of normal blood pressure was defined. The pattern, observed in random-bred CD1, inbred C57BL/6J, BALB/cJ, normoglycemic NOD and immune deficient *Rag2*<sup>-/-</sup> and *Rag2*<sup>-/-</sup>/*Il2rg*<sup>-/-</sup>, changed at specific times important for placental development. NOD *scid* and hyperglycemic NOD pregnancies differed, identifying NK cells and blood glucose values respectively as factors contributing to circulatory control over pregnancy. The precision of tel-

emetric measurement and the concordance of findings between replicate animals made 4-6 recordings sufficient to generate publication quality data. Complications and technical failures were common in these pregnancy-based studies, requiring preparation of 10-12 instrumented mice per group. These animal numbers are much lower than needed for pregnancy time course studies requiring daily euthanasia of 3-6 replicate mice. Previous attempts to collect this information using serial, daily, tail cuff recording gave data with sufficient variability that we concluded (incorrectly) mice differed from humans because they lacked a dynamic pattern of cardiovascular regulation across pregnancy.

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IV-4-097

## A novel *in vivo* approach to measure multiple organ system functions simultaneously: Combining automated sampling/delivery systems with radio telemetry (ABST)

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We describe a novel validated apparatus combining computer automated blood sampling and delivery of substances simultaneous with radio telemetry of physiological parameters in rats. For this, a unique engineering solution was created to combine the BASi Culex<sup>®</sup> automated blood sampling system with Data Sciences International Physio-Tel<sup>®</sup> Multiplus radio telemetry system. Studies were performed using Han-Wistar rats previously prepared with indwelling catheters and a radio telemetry transmitter. The system was optimized to maintain weight gain and reduce overall stress as evidenced by plasma hormone biomarkers. Parameters presently measured from a single animal include: heart rate, blood pressure, body temperature, electroencephalogram, drug exposures, urine chemistries, renal biomark-

ers of injury, and functional measures renal plasma flow and glomerular filtration rate. Using the ABST system has enabled an ~80% reduction in animal numbers required for a single test article compared with traditional stand-alone studies for each organ system. Studies performed using the ABST system to measure multiple organ functions simultaneously *in vivo* reduce the need for multiple serial studies, allow pair-wise data comparisons, enable better temporal evaluations and provide true pharmacokinetic and pharmacodynamic analyses. Employing this model early in the drug discovery process is intended to stop progression of toxic compounds and replace testing in higher mammalian species. Studies designed to exemplify system optimization and validation will be provided.

IV-4-375

## PET and MRI improve safety testing with far fewer animals (<50%)

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The new OECD guideline for an Extended One-Generation Reproduction Toxicity Study (OECD EOGRTS) aims to reduce the number of animals for REACH and other testing programs without compromising safety assessment. The EOGRTS-protocol includes reproduction, but also the developing immune and neurological systems.

Twenty rats per sex and group are required to test developmental neurotoxicology. In studies with well-known toxicants (MAM, MeHg, organotin compounds DOTC and TBTO, and ethanol) we demonstrated that this number can be reduced by about 50% using *in vivo* imaging, and prediction to humans improved when combined with micro array gene expression profiling.

Changes in brain activity (uptake patterns of [18F]FDG micro-PET imaging) were observed over time in animals with impaired behavior demonstrated by conventional means (Func-

tional Observational Battery; Motor Activity). Changes in brain region volume (MR-Imaging) concurred with brain weight and size measured after death, but was more detailed and identified more closely predilection areas for toxicity. Gene expression profiling of brain parts at young and adult age proved to corroborate an apparent delay in structural and/or functional development and/or persistent impairment, not only of the neurological system but also of the immune and endocrine systems.

Our results show that combined application of PET, MRI, and gene expression profiling warrants a strategy, resulting in improved prediction for man and substantial reduction and refinement of animal use. More imaging modalities are explored. With time this strategy can replace conventional, logistically complex and laborious animal testing in (regulatory) toxicology and safety pharmacology studies, saving animals on a large scale.



## Session IV-4: Poster presentations

IV-4-204

### Multimodal analgesia for improved recovery of dogs surgically prepared for telemetry

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Dogs are surgically implanted with DSI Physio Tel™ transmitters to produce a long term telemetry model in freely moving dogs with the ability to measure left ventricular pressure, epicardial electrocardiogram and aortic blood pressure. This is achieved by a lateral thoracotomy, which is considered to be a surgical procedure with the potential for severe pain.

A standard analgesia plan has been devised for the surgery with slight modifications in response to the analgesic needs of the individual animal. Carprofen results in long acting analgesia and anti-inflammatory action. Bupivacaine is administered via an intercostal block and gives medium-length, locally-acting analgesia in the intra-operative and post-operative period. This particularly addresses the pain caused by chest wall movement. Methadone is administered strategically pre-operatively, im-

mediately post-operatively and three hours post-operatively to coincide with the bupivacaine block wearing off. This is followed with buprenorphine, which, with carprofen, ensures good overnight analgesia. The analgesia is tailored to respond to individual pain requirements by the use of fentanyl as a short-acting analgesic with a rapid effect.

It is evident from the post-operative clinical records that it is normal for dogs to be standing and walking by 5 h, with many achieving this within 3 h. Also, the dogs are normally extremely bright and eating 24 h post-operatively.

A multimodal approach to analgesia, customised for each dog, allows us to address the individual analgesic needs of each animal resulting in smoother and more rapid recoveries from this painful procedure. This has refined welfare.

IV-4-279

### Advancing technology and the 3Rs: simultaneous pharmacokinetic and multi-organ function assessment in rats

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Innovation leading to development, qualification, and implementation of new technologies produces Refinement in data quality, Reduction in animal use, improvement in clinical safety, and reduced attrition in pharmaceutical development. We demonstrate an integrated pharmacology platform capable of simultaneous acquisition of pharmacokinetic and multiple organ system function data in the same animal. This platform using conscious rats surgically implanted with radiotelemetry transmitters and externalized cannulas allows: 1) automated blood sampling; 2) simultaneous assessment of cortical electroencephalography (EEG) or electrocardiogram (ECG); 3) cardiovascular parameters (SP, DP, MAP and HR); 4) body temperature and activity; 5) renal hemodynamics (GFR and RPF) and excretory functions (electrolytes and metabolic products), 5) assessment of biomarkers of drug-induced organ system injury (DILI, DIKI, DIVI,

etc.); 6) terminal histopathology. As a case example, single administration of cisplatin (15 mg/kg, i.p.) in Han Wistar rats (n=8) produced measurable plasma concentrations associated with decreased HR, temperature, GFR and RPF (-37%, -13%, -74%, -35% vs. control, respectively). Cisplatin also induced elevated DIKI biomarkers (fold increase over control:  $\alpha$ -GST, 76; GSTYb1, 12; clusterin, 9; albumin, 27; Kim-1, 5, respectively) correlated with histopathologic renal tubular injury by day 3. In conclusion, implementation of ABST technology collected: 1) data in one study, where traditional methods required 4 or more studies; 2) data simultaneously collected in one study group rather than traditionally collected in parallel from 4 or more groups plus separate controls, and 3) a total of 8 animals compared to a minimum of 43 animals (81% reduction).



IV-4-389

## Sexual dimorphism in brain function of DBA2 mice studied with functional and structural MRI – an animal refinement and reduction approach

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Gender related brain dimorphisms exist in all vertebrate species including humans and rodents. For investigations of brain structure and function mice are regularly used as models. As a novel, non-invasive technique magnetic resonance imaging (MRI) is capable of obtaining high resolution data of brain function and structure in humans and (anesthetized) mice. However, in the current literature only 1% of all MRI animal studies combine structural and functional high resolution MRI data acquisition for the very same specimen.

Because the structure-function relationship is important for detailed interpretation of MRI data, the aim of this study is to combine structural and functional MRI data from the same animals investigating gender differences in pain processing of DBA2 mice. Functional MRI data were obtained by repetitive thermal stimuli. After the functional experiment, the animals

had to be sacrificed due to the ethical rules. Subsequently, high resolution MRI was performed on the dead animals.

Preliminary results of structural MRI show no gender differences in the total brain volumes, in contrast to human MRI data. Principal component analysis of functional MRI data shows gender differences in pain response and threshold.

Using non-invasive MRI allows performing functional and structural high resolution MRI experiments sequentially in the same animals. Obviously this approach reduces the number of subjects needed by 50%.

This work contributes to the goal of the 3R's by means of non-invasive imaging in anesthetized animals leading to high resolution data (refinement), as well as combining structural and functional data from the same animal (reduction).

IV-4-392

## Integrative wireless monitoring for animal wellbeing

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Minipigs are recognized as an alternative non-rodent model that can improve drug safety for humans for its superior predictivity and translation to man (<http://www.rethink-eu.dk>). We hypothesized that this contribution to animal 3Rs could be further improved when deploying Holst Centre wireless sensor technology in this area of (mandatory) safety evaluation studies.

We explored – as a first initiative – an integrative application of the Holst Centre ECG Necklace sensor node combined with an X, Y and Z-acceleration sensor in minipig as the test subject. Primary focus was on 1) animal wellbeing during monitoring; 2) usefulness and quality of the signals; 3) relevance of the integrative simultaneous information of ECG, heart rate (HR) and acceleration (activity).

The results demonstrated that 1) the minipig could freely move during continuous or repeated monitoring and unambigu-

ously accepted wearing the sensor (refinement). 2) The continuous ECG, Heart rate (HR) and acceleration signals were acquired and post-analysed with very good results (reduction). 3) Changes in heart rate could be explained by changes in acceleration (due to the animal's activity) and changes in orientation (X, Y, Z-position) of the animal were observed as changes in one or more of the acceleration (X, Y, Z-) signals.

It is concluded that the Holst Centre wireless sensor technology perfectly fits in to contribute to animal refinement/reduction: continuous and repeated, simultaneous monitoring with multiple sensor nodes addressing multiple organ systems seems to be within reach and so more information can be obtained from fewer animals. Moreover, decision making during drug development is stepping up.