PROGRAMME AND ABSTRACT BOOK

IMPROVING EXPERIMENTAL APPROACHES IN ANIMAL BIOLOGY: IMPLEMENTING THE '3RS'

29 JUNE – 1 JULY 2016
CHARLES DARWIN HOUSE,
LONDON, UK

TESTING TESTING 1,2,3

SOCIETY FOR EXPERIMENTAL BIOLOGY

IMPROVING EXPERIMENTAL APPROACHES IN ANIMAL BIOLOGY: IMPLEMENTING THE 3RS

ORGANISED BY:
DR LYNNE U SNEDDON
UNIVERSITY OF LIVERPOOL, UNITED KINGDOM
& DR NIC BURY
KING'S COLLEGE LONDON, UNITED KINGDOM

SYMPOSIUM SUPPORTED BY:

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lasa Laboratory Animal Science Association

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

RSPCA

Understanding ANIMAL RESEARCH

DELEGATE INFORMATION

BADGES

Participants are required to wear name badges at all times for proof of registration, security purposes and catering identification.

CATERING

Lunch and refreshments during the meeting are included in your registration fee and will be served in the exhibition / breakout area. The Conference Dinner on Thursday 30 June will be held at The Rugby Tavern, 19 Great James St, London, WC1N 3ES. This is a 5 minute walk from Charles Darwin House.

CERTIFICATE OF ATTENDANCE

Delegates requiring a certificate of attendance should visit the SEB registration desk on their departure.

VENUE

Charles Darwin House
12 Roger Street
WC1N 2JU
United Kingdom

The scientific sessions will be taking place in the Charles Darwin Lecture Theatre on the ground floor. Exhibition and poster session will be taking place in the breakout area.

LIABILITY

Neither the Society for Experimental Biology nor Charles Darwin House will accept responsibility for damage or injury to persons or property during the meeting. Participants are advised to arrange their own personal health and travel insurance.

PHOTOGRAPHY

No photographs are to be taken of the speakers and their slides during the symposium.

**Please note: The SEB will be taking photos during the event for promotional purposes. If you have any concerns, please visit the SEB registration desk.*

POSTER SESSIONS

The poster session will be taking place in the exhibition/breakout area between **17:30-19:00 on Wednesday 29 June**. Poster presenters are invited to hang their poster on their arrival (Velcro will be provided) and asked to remove their posters by **15:30 on Friday 1 July**. Any posters left behind will be disposed of.

REGISTRATION

The registration desk will be open during the hours of the symposium and a SEB staff member will be on hand during the refreshment and lunch breaks should you require any assistance

TWITTER

We're looking to increase the conversation at the symposium using Twitter so please get tweeting! Follow the conversation by using the hashtag **#SEB3Rs**
SEB - **@SEBiology**

WI-FI INTERNET ACCESS

Internet access is available during the meeting and free of charge. The log in details are:

Network: **CDH**
Password: **time2work**

EXHIBITOR PROFILES

NC3Rs

@ ENQUIRIES@NC3RS.ORG.UK
WWW.NC3RS.ORG.UK

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is an independent scientific organisation. It supports the UK science base by driving and funding innovation and technological developments that replace or reduce the need for animals in research and testing, and lead to improvements in welfare where animals continue to be used. The Centre promotes robust and ethical scientific practice through collaborating with research funders, academia, industry, regulators and animal welfare organisations, both in the UK and internationally. The NC3Rs is supported primarily by Government, but also receives funding from the charitable and industrial sectors. It has an annual budget of approximately £10 million and is the UK's major funder of 3Rs research. Further information about NC3Rs activities and programmes can be found at **www.nc3rs.org.uk.**

NC 3Rs
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for the Replacement
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of Animals in Research

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PROGRAMME OVERVIEW

WEDNESDAY 29 JUNE

10:00
Registration and opening of exhibition

11:00
Welcome and introduction

SESSION 1: REFINEMENT
11:10
Scientific session

13:00
Lunch / exhibition

14:15
Scientific session

15:30
Refreshment break / exhibition

16:00
Scientific session

16:55
Pecha Kucha

17:30
End of Day 1

17:30-19:00
Poster session

THURSDAY 30 JUNE

08:30
Registration / exhibition

SESSION 2: REPLACEMENT
09:00
Scientific session

11:00
Refreshment break / exhibition

11:20
Scientific session

13:00
Lunch / exhibition

SESSION 3: REDUCTION

14:00
Scientific session

15:30
Refreshments / exhibition

16:00
Scientific session

17:30
End of day 2

19:00 – LATE
Conference dinner – The Rugby Tavern

FRIDAY 1 JULY

09:00
Registration / exhibition

SESSION 4: 3RS RESEARCH OPPORTUNITIES AND RESOURCES
09:15
Scientific session

10:50
Oral and Poster Prizes

10:55
Refreshment break / exhibition

11:10
Scientific session

12:40
Lunch / exhibition

SESSION 5: EDUCATING THE 3RS
13:30
Scientific session

15:30
Closing comments and end of symposium

PROGRAMME

WEDNESDAY 29 JUNE

10:00
Registration and opening of exhibition

11:40
Welcome

SPEAKERS
Dr Lynne U Sneddon
University of Liverpool, United Kingdom

Dr Nic Bury
King's College London, United Kingdom

SESSION 1: REFINEMENT
CHAIR: DR LYNNE U SNEDDON
11:10–13:00

11:10
Dr Maja Wallberg
University of Cambridge, United Kingdom
The use of non-invasive imaging to monitor immune responses in islets
REF.1

11:40
Dr Augusto Vitale
Istituto Superiore di Sanità, Italy
Refinement in the use of non-human primates in neuroscientific research
REF.2

12:00
Dr Charlotte A Hosie
University of Chester, United Kingdom
Refining the laboratory husbandry of the African clawed frog, *Xenopus laevis*
REF.3

12:20
Ms Rachel J Chance
Scottish Fish Immunology Research Centre, University of Aberdeen, United Kingdom
Developing non-lethal sampling: the effect of repeated anaesthesia on model host Atlantic salmon, *Salmo salar*, and model pathogen, *Neoparamoeba perurans*
REF.4

12:35
Dr Katherine Sloman
University of the West of Scotland, United Kingdom
Transgenerational effects of refinement in fish
REF.5

13:00 LUNCH / EXHIBITION

CHAIR: DR LYNNE U SNEDDON
14:15– 15:30

14:15
Dr Amanda CdeC Williams
University College London, United Kingdom
Improving identification of pain
REF.6

14:35
Dr Matthew Leach
Newcastle University, United Kingdom
Pain assessment using facial expressions, what do we know?
REF.7

15:05
Dr Johnny Roughan
Newcastle University, United Kingdom
Inflammation imaging for analgesic dose rate refinement in mice
REF.8

15:30 REFRESHMENT BREAK / EXHIBITION

PROGRAMME

CHAIR: DR NIC BURY
16:00–17:30

16:00
Dr Lynne U Sneddon
University of Liverpool, United Kingdom
Big brother is watching you: Automated assessment of health in fish
REF.9

16:25
Miss Lauren E James
Aarhus University, Denmark
Feeding behaviour as an indicator of pain perception in the ball python (*Python regius*)
REF.10

16:40
Miss Catherine J A Williams
Aarhus University, Denmark
The physiological effects of morphine in the South American rattlesnake *Crotalus durissus*
REF.11

16:55
Pecha Kucha

17:30 END OF DAY 1

17:30-19:00
Poster Session

THURSDAY 30 JUNE

08:30
Registration / Exhibition

SESSION 2: REPLACEMENT
CHAIR: DR NIC BURY
09:00– 11:00

09:00
Dr Gil G Rosenthal
Texas A & M University, United States
Artifice in animal behavior: breaking trade-offs among the 3R's
REP.1

09:25
Prof George Kemenes
University of Sussex, United Kingdom
The use of *Lymnaea stagnalis* as an invertebrate model system to study amyloid-induced and age-related memory impairment
REP.2

09:50
Prof Robin S B Williams
Royal Holloway University of London, United Kingdom
Investigating the pharmacogenetics of flavonoids using a 3Rs model
REP.3

10:15
Dr Ildiko Kemenes
Sussex Neuroscience School of Life Sciences, University of Sussex, United Kingdom
Evolutionary conserved mechanisms of associative learning in *Lymnaea*
REP.4

10:30
Ms Anushika P.H.M Herath
School of Biological Sciences, The University of Sydney, Australia
DNA metabarcoding for noninvasive diet analysis of herbivores using Common Brushtail Possum (Trichosurus vulpecula) as a model
REP.5

PROGRAMME

10:45
Ms Esther A Odekunle
Queen Mary University of London, United Kingdom
Molecular and neuroanatomical characterization of vasopressin/oxytocin-type signalling in an echinoderm
REP.6

11:00 REFRESHMENT BREAK / EXHIBITION

CHAIR: PROF CRAIG FRANKLIN
11:20–13:00

11:20
Dr Stefan Scholz
UFZ, Germany
Improving the predictive capacity of the fish embryo test by analysis and quantitation of AOP-linked endpoints
REP.7

11:45
Ms Melanie Knöbel
EAWAG, Switzerland
Alternatives to animal testing in ecotoxicological risk assessment
REP.8

12:10
Dr Nic Bury
Kings College London, United Kingdom
Development of an *in vitro* gill cell culture model to replace fish in chemical uptake studies
REP.9

12:30
Dr Darren M Moss
University of Liverpool, United Kingdom
The development of a physiologically based pharmacokinetic rat model for simulating absorption, distribution and elimination of environmental and pharmacological compounds
REP.10

12:45
Ms Laura M Langan
Plymouth University, United Kingdom
Morphological and metabolic characterization of the rainbow trout intestine grown *in vitro*: from pyloric to posterior
REP.11

13:00 LUNCH / EXHIBITION

SESSION 3: REDUCTION
CHAIR: DR NIC BURY
14:00–15:30

14:00
Dr Manasi Nandi
King's College London, United Kingdom
Animal models of sepsis and maximising data usage: a refinement and reduction case study
RED.1

14:30
Prof Malcolm Macleod
University of Edinburgh, United Kingdom
Rigour, Rigour, Rigour… practical approaches to improving the harm-benefit ratio in animal research
RED.2

15:00
Dr Lewis G Halsey
University of Roehampton, United Kingdom
Reduce with caution: small samples bring fickle P values and bloated effect size
RED.3

15:30 REFRESHMENT BREAK / EXHIBITION

CHAIR: DR LYNNE U SNEDDON
16:00–17:30

PROGRAMME

16:00
Prof Dominic J Wells
Royal Veterinary College, United Kingdom
Relevance is the 4th R in animal experiments
RED.4

16:30
Dr Nathalie Percie du Sert
NC3Rs, United Kingdom
NC3Rs resources to improve the design and reporting of animal research
RED.5

17:00
Prof Craig E. Franklin
Chair of the Animal Section, Society for Experimental Biology & Executive Director Research Ethics, The University of Queensland, Australia
The universality of the 3R's of animal ethics across a diverse and global research community
RED.6

17:30 END OF DAY 2

19:00-LATE
Conference Dinner - The Rugby Tavern

FRIDAY 1 JULY

09:00
Registration / Exhibition

SESSION 4: 3RS RESEARCH OPPORTUNITIES AND RESOURCES
CHAIR: DR LYNNE SNEDDON
09:15–10:55

09:15
Dr Mark J Prescott
NC3Rs, United Kingdom
Pioneering better science through the 3Rs: An introduction to the NC3Rs
EDUC.1

09:35
Ms Lydia Darragh
BBSRC, United Kingdom
Biotechnology and Biological Sciences Research Council - BBSRC
EDUC.2

09:55
Mrs Caroline Chadwick
LASA, United Kingdom
What is LASA?
EDUC.3

10:15
Ms Wendy Jarrett
UAR, United Kingdom
The importance of openness about animal research
EDUC.4

10:35
Panel discussion

10:50
Oral and Poster prizes

10:55 REFRESHMENT BREAK / EXHIBITION

CHAIR: DR NIC BURY
11:10–12:40

11:10
Dr Penny Hawkins
RSCPA, United Kingdom
Engaging with ethical review workshop
EDUC.5

12:40 LUNCH / EXHIBITION

PROGRAMME

SESSION 5: EDUCATING THE 3RS
CHAIR: PROF JOHN BRYANT
13:30–15:30

13:30
Prof John A Bryant
University of Exeter, United Kingdom
The Three Rs: developing an ethical framework
EDUC.6

14:10
Dr Chris Willmott
University of Leicester, United Kingdom
The use of multimedia in bioethics education
EDUC.7

14:50
Prof Teresa G. Valencak
University of Veterinary Medicine, Austria
Implementing 3R's: transferring student learning from the classroom to lab and field
EDUC.8

15:30
Closing comments and end of symposium

SPEAKERS
Dr Lynne U Sneddon
University of Liverpool, United Kingdom
Dr Nic Bury
King's College London, United Kingdom

POSTER SESSION WEDNESDAY 29 JUNE

Miss Catherine J A Williams
Aarhus University, Denmark
Local anaesthetic? Systemic effects of subcutaneous lidocaine in the American bullfrog - *Lithobates catebeianus*
REF.12

Dr Rasneer Bains
MRC Harwell, United Kingdom
A novel home cage monitoring system for multiply housed mice
REF.13

Miss Grace C Laws
Institute of Neuroscience Newcastle University, United Kingdom
Investigating the temporal response to a UCMS procedure and identifying hippocampal stress markers in mice
REF.14

Dr Polly M Taylor
Topcat Metrology Ltd, United Kingdom
Refinements in thermal and mechanical nociceptive threshold testing in mice
REF.15

Mr Chris J Emmans
University of Chester, United Kingdom
Behavioural and physiological measures of stress to optimise the welfare and hence scientific potential of Lake Zacapu salamander *(Ambystoma andersoni)*
REF.16

Dr Andrew M Holmes
University of Chester, United Kingdom
Non-invasive assessment of a range of enrichments on the welfare of laboratory-housed *Xenopus laevis*
REF.17

Miss Lauren E James
Aarhus University, Denmark
Alfaxalone anaesthesia in the ball python *(Python regius).* REF.18

Ms Karen Dunford
University College London, United Kingdom
Refining severity limits with a refined health monitoring system in Zebrafish
REF.19

Ms Elena Wilde
King's College London, United Kingdom
Measuring murine blood pressure: a study to understand the parameters that lead to best practice
REF.20

Dr Lynne U Sneddon
University of Liverpool, United Kingdom
Improving the welfare of rainbow trout during an aesthesia
REF.21

Dr Matthew Baron
University of Plymouth, United Kingdom
Fish on the move: Assessing welfare during transport
REF.22

Dr Ágnes Vehovszky
MTA Centre for Ecological Research Balaton Limnological Institute, Hungary
Neuronal functions inhibited by neonicotinoid insecticides in the aquatic model organism *Lymnaea stagnalis*
REP.12

Dr Richard J Maunder
Plymouth University, United Kingdom
Development and adaptation of an *in vitro* rainbow trout gill model for use as an alternative to live fish studies
REP.13

Miss Elisabeth Chang
King's College London, United Kingdom
Characterizing uptake and efflux of pharmaceuticals and PPCP using fish in vitro models
REP.14

IMPROVING EXPERIMENTAL APPROACHES IN ANIMAL BIOLOGY: IMPLEMENTING THE 3RS

REF.1 THE USE OF NON-INVASIVE IMAGING TO MONITOR IMMUNE RESPONSES IN ISLETS

WEDNESDAY 29 JUNE 2016 11:10

MAJA WALLBERG (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), ROBERT BENSON (UNIVERSITY OF GLASGOW, UNITED KINGDOM), JAMES BREWER (UNIVERSITY OF GLASGOW, UNITED KINGDOM), HERMAN WALDMANN (UNIVERSITY OF OXFORD, UNITED KINGDOM), PAUL GARSIDE (UNIVERSITY OF GLASGOW, UNITED KINGDOM), ANNE COOKE (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

@ MW394@CAM.AC.UK

Type 1 diabetes is an autoimmune disease that kills the insulin producing beta cells of the pancreas. Our laboratory studies the mechanisms that lead to this destruction, and develops protocols for therapy to cure the disease. In our work we regularly make use of experimental mice, especially the non obese diabetic (NOD) mouse. One obstacle in our work is the location of the islets inside the pancreas, making it difficult to access them to assess immune infiltration and beta cell function without killing the mouse. We have developed a protocol that allows us to image infiltration in islets before, during and after treatment with immunomodulatory agents using multiphoton microscopy on islets grafted into the pinna of the ear. We have established that islets can be grafted into the pinna of the ear, and can continue to produce insulin and glucagon there. Furthermore, infiltration into islets grafted into the pinna of the ear can be detected and recorded in vivo using multiphoton imaging. Longitudinal imaging of islets grafted into the pinna can be used to determine immune infiltration in islets before, during and after treatment with immunomodulatory substances. This has allowed us to follow the resolution of immune infiltration after treatment with aglycosyl anti-CD3 antibody, and holds great potential for future studies of immune intervention as well as beta cell differentiation and function.

REF.2 REFINEMENT IN THE USE OF NON-HUMAN PRIMATES IN NEUROSCIENTIFIC RESEARCH

WEDNESDAY 29 JUNE 2016 11:40

AUGUSTO VITALE (ISTITUTO SUPERIORE DI SANITÀ, ITALY), LUCA BONINI (ISTITUTO ITALIANO DI TECNOLOGIA, UNIVERSITÀ DI PARMA, ITALY)

@ VITALE@ISS.IT

Non-human primates (NHP) are still needed for research in neuroscience, mainly because of their phylogenetic proximity to humans. Nevertheless, this evolutionary closeness also constitutes the reason why the European legislation on the protection of animals used in scientific procedures applies a special focus on NHP. In this contribution we will discuss the application of the 3Rs Principle in NHP neuroscientific research, with special attention to the concept of 'Refinement'. Conditions can be improved to minimise the level of sufferance experienced by experimental subjects both during i) the lifetime and ii) the experimental sessions. In the first case we will report on a study on common marmosets (Callithrix jacchus), where positive interactions with staff improved the general level of welfare of the colony, decreasing anti-predator behaviours and aggressiveness towards humans. In the second case, we will illustrate a new methodology aimed at recording neuronal activity from unrestrained macaques (Macaca mulatta) using wireless multielectrode systems, which allow to i) increase the quality and quantity of simultaneously recorded data, ii) reduce the number of experimental sessions needed and iii) avoid behavioural restrictions, hence improving animal welfare and ecological validity of the experimental results. For both cases, we will argue how improving the welfare of experimental animals also improves the quality of the collected data.

REF.3 REFINING THE LABORATORY HUSBANDRY OF THE AFRICAN CLAWED FROG, *XENOPUS LAEVIS*

WEDNESDAY 29 JUNE 2016 12:00

CHARLOTTE A HOSIE (UNIVERSITY OF CHESTER, UNITED KINGDOM), ANDREW M HOLMES (UNIVERSITY OF CHESTER, UNITED KINGDOM), CHRISTOPHER J EMMANS (UNIVERSITY OF CHESTER, UNITED KINGDOM), ROBERT COLEMAN (UNIVERSITY OF CHESTER, UNITED KINGDOM), TESSA E SMITH (UNIVERSITY OF CHESTER, UNITED KINGDOM)

@ L.HOSIE@CHESTER.AC.UK

Herpetologists widely acknowledge that amphibians are highly sensitive vertebrates but that assessment of their welfare has been severely neglected. Despite the use of 100,000s annually in laboratories worldwide there is little consensus on *Xenopus laevis* husbandry conditions that maximise good welfare. Guidelines exist but are underpinned by little reliable science. With NC3Rs funding we have fully validated sensitive, precise and robust endocrine techniques to measure the amphibian "stress hormone" (corticosterone) for this species and developed detailed, reliable measures to assess behaviour. Taken together (as is often exhorted in welfare science but rather little practised) these have provided a highly rigorous and reliable approach to identifying laboratory conditions that maximise welfare standards but that are "workable" for laboratories. Our results have demonstrated, for example, significant elevation of corticosterone during and after transportation but that this varies with sex. Settling into a new laboratory environment is also clearly defined by significant changes in corticosterone. Behavioural measures show agreement with the endocrine results. We are combining our behavioural and endocrine approaches to develop a Behavioural Stress Score for this species - for quick and easy use in laboratories. An overview of this project and key significant findings will be discussed. Clearly animals kept in 'best' conditions should be healthier, provide more uniform laboratory material and generate better quality results (perhaps also using fewer animals). Our results will enable significant refinement of *Xenopus laevis* husbandry and improve the lives of a large number of animals.

REF.4 DEVELOPING NON-LETHAL SAMPLING: THE EFFECT OF REPEATED ANAESTHESIA ON MODEL HOST ATLANTIC SALMON, *SALMO SALAR*, AND MODEL PATHOGEN, *NEOPARAMOEBA PERURANS*

WEDNESDAY 29 JUNE 2016 12:20

RACHEL J CHANCE (SCOTTISH FISH IMMUNOLOGY RESEARCH CENTRE UNIVERSITY OF ABERDEEN, UNITED KINGDOM), CHRISTOPHER J SECOMBES (SCOTTISH FISH IMMUNOLOGY RESEARCH CENTRE UNIVERSITY OF ABERDEEN, UNITED KINGDOM), BERTRAND COLLET (MARINE SCOTLAND SCIENCE, UNITED KINGDOM), CATHERINE COLLINS (MARINE SCOTLAND SCIENCE, UNITED KINGDOM)

@ RO1RC14@ABDN.AC.UK

Current methodology for investigating pathogen-induced immune responses in fish relies upon lethal sampling of different individuals at set time points. By implementing novel non-lethal sampling methodology, there is a potential 80-90% reduction in experimental fish needed for ectoparasite challenge experiments. Non-lethal sampling will also provide more conclusive results by improving the linking of response dynamics and infection outcome; a reduction in host, parasitic load and localised response variability; prevention of masking of patterns; and increased statistical robustness. The pathogen selected to act as a model for the design of this methodology is *Neoparamoeba perurans*, the etiological agent of amoebic gill disease (AGD), a globally important ectoparasite of farmed fish. Initial work has focused on assessing whether repeated exposure to three fish anaesthetics, AQUI-S®, MS-222 and metomidate, at the same concentration and duration required to anaesthetise post-smolt Atlantic salmon to Stage 4 anaesthesia, would have a deleterious effect upon the viability and growth rate of *in vitro* cultures of *N. perurans*. *In vivo* experiments twinned with the *in vitro* work were designed to assess the effect of repeated induction to Stage 4 anaesthesia compared to the current lethal anaesthesia overdose method of sampling upon gill and general fish health. A novel in-tank anaesthetising route was utilised to reduce stress during the procedures. Results to be presented will include gill health indicators and head kidney stress gene expression, blood-glucose assessment, peripheral leukocyte ratios and liquid chromatography mass spectrometry detection of accumulation of anaesthetics in the muscle tissue.

REF.5 TRANSGENERATIONAL EFFECTS OF REFINEMENT IN FISH

WEDNESDAY 29 JUNE 2016 12:35

KATHERINE SLOMAN (UNIVERSITY OF THE WEST OF SCOTLAND, UNITED KINGDOM), P TAMILSELVAN (UNIVERSITY OF THE WEST OF SCOTLAND, UNITED KINGDOM), L EATON (UNIVERSITY OF THE WEST OF SCOTLAND, UNITED KINGDOM)

@ KATHERINE.SLOMAN@UWS.AC.UK

There is increasing evidence that the conditions a mother experiences can have significant effects on the behaviour and physiology of her offspring. We have shown that maternal stress as mild as alterations in routine husbandry practice can be significant enough to alter the behavioural phenotype of offspring. Additionally, stress experienced by mothers generates greater levels of individual variation in behaviour. We have also found that holding fish in either environmentally-enriched or non-enriched tanks alters activity and anxiety behaviours in their offspring. Therefore, when refining ways animals are held in captivity, it is important to understand how husbandry procedures can affect both the animal and its progeny and to be aware that parental experiences can alter variability in offspring performance.

REF.6 IMPROVING IDENTIFICATION OF PAIN

WEDNESDAY 29 JUNE 2016 14:15

AMANDA CdeC WILLIAMS (UNIVERSITY COLLEGE LONDON, UNITED KINGDOM)

@ AMANDA.WILLIAMS@UCL.AC.UK

Spontaneous behaviours indicating pain, described in identifying welfare problems in animals, are underused in analgesic testing and other pain science studies, often dominated by evoked pain tests. The recent identification of facial expression of pain in lab mice and rats and in some larger mammals provides a subtle sign of pain, and one sensitive to social context. Recent work with fish and in cephalopods and crustaceans suggests flexible and effective behaviours associated with pain, driven by central sensitisation, and abolished by analgesia. These studies can help us to develop classifications of pain-related behaviours, and their functions, and help to fill some of the gaps in our understanding of how and under what circumstances animals express pain or suppress its expression. All these can contribute to better detection and measurement of pain, contributing to the refinement and reduction agenda. I explore how an evolutionary framework provides a basis for such a classification, and how it directs attention to particular behaviours and to ecologically valid pain stimuli.

REF.7 PAIN ASSESSMENT USING FACIAL EXPRESSIONS, WHAT DO WE KNOW?

WEDNESDAY 29 JUNE 2016 14:35

MATTHEW LEACH (NEWCASTLE UNIVERSITY, UNITED KINGDOM), AMY MILLER (NEWCASTLE UNIVERSITY, UNITED KINGDOM), PAUL FLECKNELL (NEWCASTLE UNIVERSITY, UNITED KINGDOM)

@ MATTHEW.LEACH@NEWCASTLE.AC.UK

The assessment of pain in non-human animals remains difficult but not impossible. It is argued the major limitation in the effective assessment of pain in animals is their inability to directly communicate their pain those who care for them. This limitation is not only faced by animals but also by various groups of non-communicative humans. It was suggested over 30 years ago by Grunau and Craig that the approaches used to assess pain in non-verbal humans could form a framework for pain assessment in animals. In humans, the facial expressions exhibited in response to pain are used as the gold standard form of assessment, as they are considered to be effective, accurate, easy and rapid to carry out. Recently, facial expressions scales ('Grimace Scales') have been developed to assess pain in animals, including rodents, rabbits and horses. These scales have been demonstrated (to varying degrees) to respond to painful stimuli and that these responses are ameliorated by the administration of effective pain relief. However, before the scales can be used clinically to assess pain and the effectiveness of pain relieving drugs that we administer they must be shown to be practical, repeatable and above all valid. In this presentation I will discuss whether the current scales meet these criteria and what questions remain unanswered. This will include whether these scales can be used to assess post-procedural pain, whether they are pain-specific or simply pain-related, and the current their current limitations.

REF.8 INFLAMMATION IMAGING FOR ANALGESIC DOSE RATE REFINEMENT IN MICE

WEDNESDAY 29 JUNE 2016 15:05

JOHNNY ROUGHAN (NEWCASTLE UNIVERSITY, UNITED KINGDOM), HENRI BERTRAND (NEWCASTLE UNIVERSITY, UNITED KINGDOM), JOSHUA GRIMES (NEWCASTLE UNIVERSITY, UNITED KINGDOM), HANNAH ISLES (NEWCASTLE UNIVERSITY, UNITED KINGDOM)

J.V.ROUGHAN@NCL.AC.UK

Non-steroidal analgesics are commonly used in laboratory rodents, mainly on the assumption that their post-operative pain-preventative effects align with their anti-inflammatory capabilities. Amongst many testing options, effective dose rates can be derived by demonstrating reduced post-surgical behavioural and body-weight alterations. However, in most cases NSAIDs appear to be less effective in mice than in rats; possibly because they have less potent anti-inflammatory actions or because detecting their pain-relieving effects is more difficult in mice. Our group therefore undertook a series of investigations to evaluate the anti-inflammatory versus pain-preventative properties of meloxicam given prior to laparotomy in BALB/c mice. A fluorescent probe was used to monitor pro-inflammatory COX-2, a bioluminescent probe to determine wound macrophage infiltration, and pain was assessed using behaviour assessments and the Mouse Grimace Scale (MGS) both 'live' and by the usual approach of scoring photographs. The handling methods shown by Hurst et al. (2010) to reduce anxiety were also tested for their refinement potential. Although all meloxicam dose rates (1-20mg/kg) reduced inflammation, persistent behavioural alterations and elevated MGS scores suggested none prevented pain. However, 'cupping' or tunnel handling as opposed to the usual method of tail handling mice caused a pronounced reduction in pain-specific behaviours. The time spent acclimating mice this was the key factor underpinning this seemingly effective non-pharmacological method of minimising post-surgical pain. Results will be discussed with regard to possible reasons why mice seem to be unresponsive to NSAIDs, along with the refinement possibilities offered by this simple alteration to standard mouse husbandry procedures.

REF.9 BIG BROTHER IS WATCHING YOU: AUTOMATED ASSESSMENT OF HEALTH IN FISH

WEDNESDAY 29 JUNE 2016 16:00

LYNNE U SNEDDON (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

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Fish are subject to laboratory procedures that may give rise to pain and stress thereby affecting their health. However, the scientific community have no means of rapidly and accurately identifying health status making it particularly difficult to assure laboratory fish welfare. As a significant model species in research, the numbers of zebrafish used in experiments grows globally, thus an automated system to gauge health would be a major step forward. The impact of a variety of treatments was investigated on individual zebrafish to enable the design and prototyping of a novel, automated vision-based fish health monitoring system (FMHI). It combines two key movement-based derived parameters, activity and distance travelled, into a single health index for the subject, applied over several overlapping timescales. Our results, including blind testing of novel treatments, demonstrate the FMHI can accurately detect health in zebrafish from healthy through to abnormal, providing the basis for real-time alerts. From an ethical and 3Rs perspective, the FHMI represents a significant refinement in the use of fish models in experimentation.

REF.10 FEEDING BEHAVIOUR AS AN INDICATOR OF PAIN PERCEPTION IN THE BALL PYTHON *(PYTHON REGIUS)*

WEDNESDAY 29 JUNE 2016 16:25

LAUREN E JAMES (AARHUS UNIVERSITY, DENMARK), CATHERINE JA WILLIAMS (AARHUS UNIVERSITY, DENMARK), MADS F BERTELSEN (COPENHAGEN ZOO, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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The use of reptiles in comparative physiology is well established, however it remains challenging to assess whether a reptile is in pain as a result of recognised experimental protocols. Using withdrawal reflexes and physiological responses to indicate pain perception and

to test the efficacy of analgesic agents has thus far led to inconclusive results, particularly in snakes. To refine experimental procedures on reptiles, objective methods of pain monitoring and management are key. Here, we present the potential use of routine feeding behaviour as an adjunct to current pain assessment protocols. Feeding is easily and habitually monitored in both clinical and research environments, thus providing an optimal behaviour to investigate. The aim of this study was to examine whether chemical (capsaicin injection) or physical (surgial incision) noxious stimulation would elicit a delay in feeding behaviour in the ball python (*Python regius*), a snake frequently used in physiological research. The administration of anaesthesia alone had minimal effect on feeding, whereas normal feeding did not resume until 1 and 3 weeks later following a chemical (remote capsaicin injection) or a surgical (sham catheter placement surgery) stimulus, respectively. The surgial stimulus significantly affected feeding behaviour (p=0.01), and when a different group of animals was subjected to the same stimulus, with local anaesthesia (bupivacaine 2mg/kg), this alteration to feeding behaviour was significantly reduced (p=0.006). These findings demonstrate a delay in feeding behaviour as a potential indicator of pain perception in snakes, and future work investigating the efficacy of analgesia using this model shows promise.

REF.11 THE PHYSIOLOGICAL EFFECTS OF MORPHINE IN THE SOUTH AMERICAN RATTLESNAKE *CROTALUS DURISSUS*

WEDNESDAY 29 JUNE 2016 16:40

CATHERINE J A WILLIAMS (AARHUS UNIVERSITY, DENMARK), LAUREN E JAMES (AARHUS UNIVERSITY, DENMARK), CLEO A C LEITE (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL), DIANA MONTEIRO (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL), MADS F BERTELSEN (CENTRE FOR ZOO AND WILD ANIMAL HEALTH COPENHAGEN ZOO, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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Although morphine represents the gold-standard for analgesia in mammals, an effective opioid remains to be demonstrated in snakes; no reliable anti-nociceptive effect was reported for thermal nociceptive stimulation in corn snakes, and morphine does not appear to exert significant analgesia upon subcutaneous capsaicin injections in ball pythons. Here we report the physiological effects of morphine in *Crotalus durissus*; the South American Rattlesnake. Arterial catheters were placed under isoflurane anaesthesia and local bupivacaine with either intramuscular morphine at 10 mg kg^{-1} or saline. Catheters allowed determination of heart rate, mean arterial blood pressure and plasma corticosterone concentration. Morphine administration at induction caused a tendency towards tachycardia throughout surgery and recovery. Corticosterone concentration also tended to be higher in the morphine-treated snakes (morphine, 0 hr [corticosterone] 479 ± 187 ng ml^{-1}, 48 hr [corticosterone] 410 ± 175 ng ml^{-1}), while the control group showed the expected tendency for postoperative decrease in corticosterone concentrations (control, 0 hr [corticosterone] 410 ± 175 ng ml^{-1}, 48 hr [corticosterone] 211 ± 121 ng ml^{-1}). There was a significant tachycardia in snakes when morphine was administered post operatively; with heart rates of 38 ± 11 beats min^{-1} in morphine and 22 ± 7 beats min^{-1} in control snakes at 7 hours after intramuscular administration. This corroborates previous findings in ball pythons. In conclusion, morphine at 10 mg kg^{-1} did not reduce heart rate or plasma corticosterone in South American rattle snakes when administered pre-operatively, and was associated with a significant tachycardia when administered at rest.

REP.1 ARTIFICE IN ANIMAL BEHAVIOR: BREAKING TRADE-OFFS AMONG THE 3R'S

THURSDAY 30 JUNE 2016 09:00

GIL G ROSENTHAL (TEXAS A & M UNIVERSITY, UNITED STATES), DANIEL LEE POWELL (TEXAS A & M UNIVERSITY, UNITED STATES)

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Artificial stimuli, from painted dummies and recorded calls to synthetic animations and robots, play a fundamental role in the experimental study of animal behavior. New technologies like computer animation allow for scientific rigor in manipulating signals (refinement), without having to manipulate live animals (reduction and replacement). These advantages are counterbalanced by some important deficiencies of artificial signals, such as the absence of a polarization signal or one in the ultraviolet, and by the need to sample live animals in order to construct stimuli. For studies using artifice to be interpretable,

they must be couched in a broader experimental design that in practice involves sampling more individuals and/or running a greater number of tests per individual, and attending carefully to social, habitat, and nutritional requirements. Used thoughtfully, artifice can therefore eliminate need to manipulate animals to produce signals, serve as an analytical tool for understanding how animals interact socially and with their environment, and provide a powerful incentive for experimenters to contemplate their approach to animal care and testing.

REP.2 THE USE OF *LYMNAEA STAGNALIS* AS AN INVERTEBRATE MODEL SYSTEM TO STUDY AMYLOID-INDUCED AND AGE-RELATED MEMORY IMPAIRMENT

THURSDAY 30 JUNE 2016 **09:25**

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The last common ancestor of snails and humans lived more than 600 million years ago but despite the obvious differences in body design and behaviour there is a remarkable level of conservation between the molecular mechanisms underlying associative learning in snail and man. Prime examples of this conservation are the key roles the same signaling molecules (e.g., cAMP), kinase enzymes (e.g., PKA, CaMKII) and transcription factors (e.g., CREB, C/EBP) play in learning and memory in both the molluscan and the human brain; or how the same molecules (e.g., amyloid peptides) wreak havoc on memory in both snails and humans. We investigate such conserved mechanisms of memory function and dysfunction in the pond snail *Lymnaea stagnalis*, a highly advantageous experimental system for the combined use of behavioural, electrophysiological and molecular methods in a top-down approach to the analysis of learning and memory. Using this model system we recently elucidated the pre-apoptotic neuronal mechanisms of how consolidated associative memory in *Lymnaea* is disrupted by oligomeric amyloid beta peptides. We also established that a molluscan homologue of the mammalian Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is both necessary and instructive for long-term memory formation in *Lymnaea*. Moreover, we found that PACAP expression is lower in aged compared to young *Lymnaea* and this is correlated with age-related memory impairment. Remarkably, systemic treatment of aged *Lymnaea* with PACAP reversed age-related memory deficiency. This invertebrate model system can therefore provide valuable insights into both the most fundamental mechanisms and possible treatments of memory disorders.

REP.3 INVESTIGATING THE PHARMACOGENETICS OF FLAVONOIDS USING A 3Rs MODEL

THURSDAY 30 JUNE 2016 **09:50**

ROBIN S B WILLIAMS (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

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Flavonoids comprise a large group of polycyclic compounds that are present in a range of fruits and vegetables. Diets high in flavonoids have been widely acknowledged to provide significant health benefits, yet a direct role for flavonoids in health remains controversial. Understanding the cellular role of flavonoids thus remains a high priority for dietary-related improvements in health. Animal models are considered the standard approach for flavonoid research, employing *in vitro* and *in vivo* approaches to elucidate their targets and mechanisms of action. We have developed and validated an unusual 3Rs model for flavonoids research - the social amoeba *Dictyostelium discoideum*. This model enables the rapid screening of mutant libraries to identify proteins controlling the effect of flavonoids, and the characterisation of their molecular and cellular effects. We have demonstrated the successful use of this model with several distinct categories of flavonoids, including the flavanone naringenin, with the identification of a putative target protein in *Dictyostelium* and the confirmation that this protein is also a target in mammalian models. We also show the suitability of the model to investigate isoflavonoids and flavans. *Dictyostelium* therefore provides advantageous 3Rs model for understanding flavonoid action, and following rapid recapitulation using a small number animal models, significant advances can be made in understanding flavonoid effects at a cellular level.

REP.4 EVOLUTIONARY CONSERVED MECHANISMS OF ASSOCIATIVE LEARNING IN *LYMNAEA*

THURSDAY 30 JUNE 2016 **10:15**

ILDIKO KEMENES (SUSSEX NEUROSCIENCE SCHOOL OF LIFE SCIENCES UNIVERSITY OF SUSSEX, UNITED KINGDOM), MICHAEL CROSSLEY (SUSSEX NEUROSCIENCE SCHOOL OF LIFE SCIENCES UNIVERSITY OF SUSSEX, UNITED KINGDOM), FREDERICK LORENZETTI (SUSSEX NEUROSCIENCE SCHOOL OF LIFE SCIENCES UNIVERSITY OF SUSSEX, UNITED KINGDOM), MICHAEL O'SHEA (SUSSEX NEUROSCIENCE SCHOOL OF LIFE SCIENCES UNIVERSITY OF SUSSEX, UNITED KINGDOM), PAUL R BENJAMIN (SUSSEX NEUROSCIENCE SCHOOL OF LIFE SCIENCES UNIVERSITY OF SUSSEX, UNITED KINGDOM)

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The pond snail, *Lymnaea stagnalis* provides highly valuable experimental models for top-down analyses of associative learning and memory. Just like in higher vertebrates, classical and operant conditioning paradigms can be used in *Lymnaea* and the cellular and molecular mechanisms of consolidation, maintenance, retrieval, reconsolidation and forgetting of associative memory can be investigated. Long-term memory (LTM) forms after multi-trial reward and aversive conditioning but unusually, also after single-trial reward conditioning ('flash-bulb memory'). Since the single-trial conditioning only takes 2 minutes, cellular and molecular events following memory acquisition can be detected with high temporal resolution. By using this advantage of the *Lymnaea* model system, recently we have identified brief periods during consolidation when the memory goes through labile stages allowing for 'updating' the memory trace and altering it taking into account recent and more relevant experience. Reports of such lapses during the early stages of memory formation are widespread and they have been observed in many other invertebrate and vertebrate species, including humans but their function is not well understood. Our recent work has provided evidence that lapses are a general feature of consolidation and we also show that memory replacement as a form of updating can happen during lapses. A cellular and molecular level understanding of how memory is replaced during its consolidation in a tractable invertebrate model system may inform future translationally-oriented research aimed at developing new therapeutic approaches to memory dysfunctions, such as recurring memories for highly traumatic events, a debilitating symptom of Post-Traumatic Stress Disorder (PTSD).

REP.5 DNA METABARCODING FOR NONINVASIVE DIET ANALYSIS OF HERBIVORES USING COMMON BRUSHTAIL POSSUM (TRICHOSURUS VULPECULA) AS A MODEL

THURSDAY 30 JUNE 2016 **10:30**

ANUSHIKA P.H.M HERATH (SCHOOL OF BIOLOGICAL SCIENCES THE UNIVERSITY OF SYDNEY, AUSTRALIA), KATIE ROBINSON (SCHOOL OF BIOLOGICAL SCIENCETHE UNIVERSITY OF SYDNEY, AUSTRALIA), CLARE MCARTHUR (SCHOOL OF BIOLOGICAL SCIENCES THE UNIVERSITY OF SYDNEY, AUSTRALIA)

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A wide range of wildlife research necessitates the capture, handling and manipulation of free ranging animals. However, these procedures may increase their risk of injury, impairment, or mortality. Widely used diet analysis methods involve handling or rely on use of stomach content analysis. Most commonly used non intrusive method is faecal micro-histological analysis, however its use is limited by low taxonomical resolution and incomplete coverage of species consumed. Metabarcoding of fecal DNA is an emerging non-invasive diet analysis approach. No information is available on effectiveness of this method to determine the diet of marsupial herbivores in Australia.

We adapted a faecal DNA metabarcoding method to determine the diet of herbivorous Australian marsupials, using the common brushtail possum as a model. Plant DNA was extracted from 66 scat samples from 26 different individuals, collected from Ku-ring-gai Chase National Park, NSW, Australia. Plant species consumed were identified by metabarcoding of chloroplast DNA P6 loop regiontrnLandintergenic spacer regionsrpl2-trnH.

Our preliminary data reveals that DNA metabacoding method has the potential to detect more species consumed compared to micro histological analysis. Results indicate frequent consumption of family Myrtaceae species such as Eucalyptus sp Angophora sp, grass species and domestic species such as Prunus persica. Results indicate that DNA metabarcoding method has the potential as an effective non-invasive diet analysis method with high taxonomical resolution and coverage, which can be used to determine the diet of herbivorous marsupials. Using DNA metabarcoding for dietary studies may reduce the need of capturing live animals for diet determination.

REP.6 MOLECULAR AND NEUROANATOMICAL CHARACTERIZATION OF VASOPRESSIN/OXYTOCIN-TYPE SIGNALLING IN AN ECHINODERM

THURSDAY 30 JUNE 2016 **10:45**

ESTHER A ODEKUNLE (QUEEN MARY UNIVERSITY OF LONDON, UNITED KINGDOM), DEAN C SEMMENS (QUEEN MARY UNIVERSITY OF LONDON, UNITED KINGDOM), SUSAN E SLADE (UNIVERSITY OF WARWICK, UNITED KINGDOM), JAMES H SCRIVENS (UNIVERSITY OF WARWICK, UNITED KINGDOM), MICHAELA EGERTOVÁ (QUEEN MARY UNIVERSITY OF LONDON, UNITED KINGDOM), MAURICE R ELPHICK (QUEEN MARY UNIVERSITY OF LONDON, UNITED KINGDOM)

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Vasopressin/Oxytocin (VP/OT)-type peptides are a bilaterian family of neuropeptides that exert effects via co-evolved G-protein coupled receptors. Studies on vertebrates and protostomian invertebrates have revealed roles for VP/OT signalling in osmoregulation, reproduction and social behaviour. However, little is known about VP/OT-type signalling in deuterostomian invertebrates that occupy an 'intermediate' position in animal phylogeny. We have identified a VP/OT-type neuropeptide (asterotocin) in the starfish Asterias rubens by cloning a cDNA encoding its precursor and detection of the mature neuropeptide in nerve extracts using LC-MS-MS. We have also identified an A. rubens VP/OT-type receptor that is activated by asterotocin when heterologously expressed in CHO cells. Using mRNA in situ hybridization and immunocytochemistry (with novel antibodies), analysis of the expression of asterotocin and its receptor in A. rubens revealed expression in the ectoneural epithelial layer of the circumoral nerve ring and radial nerve cords, with stained processes in the underlying neuropile. Asterotocin-expressing cells were also observed in the tube feet, body wall and cardiac stomach, and immunostained processes are present in the basal nerve ring of the tube foot, the sub-epithelial nerve plexus of the body wall and the basiepithelial nerve plexus of the cardiac stomach. Consistent with the expression of asterotocin and its receptor in the cardiac stomach, pharmacological studies reveal that asterotocin triggers cardiac stomach relaxation (*in vitro*) and eversion (*in vivo*) in starfish. Furthermore, our data indicate that asterotocin may exert these effects by triggering neural release of another signaling molecule, which then acts as a muscle relaxant.

REP.7 IMPROVING THE PREDICTIVE CAPACITY OF THE FISH EMBRYO TEST BY ANALYSIS AND QUANTITATION OF AOP-LINKED ENDPOINTS

THURSDAY 30 JUNE 2016 **11:20**

STEFAN SCHOLZ (UFZ, GERMANY)

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Embryonic stages of fish are considered to sentinel less pain or stress and are therefore accepted as alternatives to testing of (adult) animals for the hazard assessment of chemicals. Hence, the use of embryos addresses the 3R principles. Given that fish embryos represent a complex organismal system they provide various advantages over the use of genuine *in vitro* cellular system and enable to study an array of endpoints that would be difficult to address in other experimental or computational alternative approaches. Most advanced at present is the prediction of acute fish toxicity using fish embryos (OECD TG 236). There has been concern that certain mode of action exhibit a weaker sensitivity but it has also shown that this weakness can be overcome by including endpoints related to the mode of action of a chemical, e.g. by the analysis of behaviour (embryonic movement).

Particularly embryos of the zebrafish provide a very versatile tool that enable to study diverse AOP (adverse outcome pathway)-linked endpoints that would allow to extend the predictive capacity to other endpoints of regulatory concern such as chronic fish toxicity, developmental toxicity or endocrine disruption. The presentation will highlight various AOP-related endpoints in the zebrafish embryo model and how their analysis and quantitation can be used beyond the assessment of acute toxicity. A focus will also be given on recent technological advances that enable also users without computer programming skills to establish routines for automatic feature assessment.

REP.8 ALTERNATIVES TO ANIMAL TESTING IN ECOTOXICOLOGICAL RISK ASSESSMENT

THURSDAY 30 JUNE 2016 **11:45**

MELANIE KNÖBEL (EAWAG, SWITZERLAND), LU TAN (EAWAG, SWITZERLAND), JULITA STADNICKA-MICHALAK (EAWAG, SWITZERLAND), KRISTIN SCHIRMER (EAWAG, SWITZERLAND)

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The main purpose of ecotoxicological risk assessment is to provide information on the concentrations of chemicals that cause an effect in organisms of different trophic levels and over short and long exposure times. Fish are the dominant vertebrate species for the regulatory evaluation of ecotoxicity of chemicals and are generally afforded the same legal protection as mammals. Each year millions of fish have been used in ecotoxicity testing. From the perspectives of the 3Rs, and for ethical but also economic reasons, the development of alternative approaches to *in vivo* fish toxicity testing is a priority. One very promising alternative approach is the use of *in vitro* cell assays, especially if they are strategically combined with computational approaches to reflect general processes in fish. In this talk we will outline several representative methods that we have developed: (i) using fish cells to predict the *in vivo* fish acute toxicity, including the results of an international round-robin study to explore the intra- and inter-laboratory comparability of the fish-cell based assay; (ii) applying fish cells in conventional cytotoxicity assays and cell impedance methods to test water effluent samples; (iii) predicting impact of chemicals on fish growth based on fish cell population growth. These approaches and results support the use of fish cells in various applications, advocate the 3Rs principles, and are promising steps towards alternatives to fish toxicity testing.

REP.9 DEVELOPMENT OF AN *IN VITRO* GILL CELL CULTURE MODEL TO REPLACE FISH IN CHEMICAL UPTAKE STUDIES

THURSDAY 30 JUNE 2016 **12:10**

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Within the European Union the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) regulations require companies to re-evaluate the environmental risk posed by the chemicals they produced. One aspect of this regulation is to determine the propensity for a compound to bioaccumulate in fish. The OECD305 test 'Bioaccumulation in Fish: Aqueous and Dietary Exposure' guidelines uses 80 fish per test, can last up to 6 months and cost many thousands of Euros. However, recent refinement sees shorter preliminary studies that use 20 fish. With 150,000 compounds registered with ECHA, of which it is estimated 3000 may require reassessment of their bioaccumulative properties, it is desirable to identify alternative *in vitro* methods. In its simplest form bioaccumulation of a parent compound is governed by the rate of uptake rate versus the rate of excretion. The gills are the site of uptake of compounds soluble in water. We have developed a primary gill cell culture that includes the different cell types found in intact gills and when grown on membrane supports can tolerate the application of water to the apical surface. The system has been used to study the uptake of various pharmaceuticals and organic compounds as well as the study of the expression of genes encoding Phase 1 metabolising enzymes and the metabolism of parent compound. Results show that *in vitro* system could act as a screen to assess the ability of chemicals to be taken up by fish - the first stage of bioaccumulation.

REP.10 THE DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC RAT MODEL FOR SIMULATING ABSORPTION, DISTRIBUTION AND ELIMINATION OF ENVIRONMENTAL AND PHARMACOLOGICAL COMPOUNDS

THURSDAY 30 JUNE 2016 **12:30**

DARREN M MOSS (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), RAJITH RAJOLI (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), ANDREW OWEN (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), MARCO SICCARDI (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

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Rodents are used extensively to investigate compound distribution, toxicity and pharmacological effects. However, published data demonstrate that, in many cases, pharmacokinetics and toxicokinetics determined in animals do not effectively predict observed data in humans. An alternative approach for predicting drug disposition is the use of physiologically based pharmacokinetic (PBPK) modelling. PBPK modelling is a bottom-up technique which aims to simulate drug distribution by combining system data describing an animal of interest (e.g. physiology, anatomy, genetics) with compound data (e.g. Caco-2 permeability, protein binding, intrinsic clearance, lipophilicity) through mathematical descriptions of absorption, distribution, metabolism and elimination (ADME). The aim of this research was to develop a rat PBPK model which includes physiologically relevant data on gut absorption, tissue composition and volume, and blood flow. Plasma concentrations of midazolam were predicted following intra-gastric administration in simulated male Sprague Dawley rats (15mg/kg, n = 4, 4 hours). The PBPK model generated accurate midazolam plasma concentrations in simulated rats when compared to *in vivo* data (C_{max} 113ng/mL versus 114ng/mL, $AUC_{0\text{-}4hr}$ 271ng/mL.hr versus 237ng/mL.hr, T_{max} 1 hour versus 0.5 hours, clearance 4.7L/hr/kg versus 4.1L/hr/kg). The rat PBPK model can be utilised for several applications: optimising experimental design to reduce rodent use; bridging from pre-clinical species to humans by taking into account physiological and anatomical differences between species; comparing predicted environmental and pharmacological compound exposures between animal species and humans, reducing the use of animals for allometric scaling.

REP.11 MORPHOLOGICAL AND METABOLIC CHARACTERIZATION OF THE RAINBOW TROUT INTESTINE GROWN IN VITRO: FROM PYLORIC TO POSTERIOR

THURSDAY 30 JUNE 2016 **12:45**

LAURA M LANGAN (PLYMOUTH UNIVERSITY, UNITED KINGDOM), STEWART F OWEN (ASTRAZENECA, UNITED KINGDOM), SIMON F JACKSON (PLYMOUTH UNIVERSITY, UNITED KINGDOM), WENDY M PURCELL (PLYMOUTH UNIVERSITY, UNITED KINGDOM), AWADHESH N JHA (PLYMOUTH UNIVERSITY, UNITED KINGDOM)

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The present study demonstrates that the gastrointestinal tract of rainbow trout *Oncorhynchus mykiss* can be isolated by a modified enzymatic dissociation protocol and maintained for up to 5 weeks in culture. Histological analysis of tissue post-digestion demonstrates extracted cells are composed of epithelium cells with minimal digestion of the laminia propria. Cell viability is consistently above 90% but can rise to 97%. The cultured epithelial cells retain their island like morphology for approximately 3-7 days (dependent on intestinal region), before gradually spreading into a flattened formation. Histological staining for the presence of mucolytic cells and tight junction formation (Z0-1, e-cadherin and f-actin) shows strong staining for neutral mucosubstances and tight junction development in all regions of the intestine. Tight junction formation was measured quantitatively using TEER and indicated a comparable resistance in transwell insert cups to those reported in the literature for fish intestine. Finally, metabolic activity assessed through the EROD assay for CYP1A activity and ECOD assay for CYP3A were assessed on all regions of the intestine and showed significant differences between regions for both assays. The maintenance and the propagation of the gut model provide compelling evidence that for the first time, fish usage during short term aquatic/dietary exposure studies can be significantly minimized in line with ethical and legal considerations. The successful culturing of all regions of the intestine will facilitate a wider range of environmental toxicants to be tested, in addition to providing a more comprehensive co-culture system to investigate toxicants with complex multi-organ metabolism.

RED.1 ANIMAL MODELS OF SEPSIS AND MAXIMISING DATA USAGE: A REFINEMENT AND REDUCTION CASE STUDY

THURSDAY 30 JUNE 2016 **14:00**

MANASI NANDI (KING'S COLLEGE LONDON, UNITED KINGDOM), PHILIP ASTON (UNIVERSITY OF SURREY, UNITED KINGDOM)

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Sepsis is the leading cause of mortality in intensive care accounting for 40,000 deaths per annum in the UK alone. It can affect anyone ranging from neonates to the elderly and antimicrobial resistance and an aging population mean that sepsis mortality is set to rise - representing a high priority area of research. Modelling sepsis in animals usually constitutes moderate to severe suffering and, although less common the UK, death is often used as an endpoint to mirror the end points in the clinic.

Sepsis can rapidly progress to a state of cardiovascular collapse, with profound hypotension and hypoperfusion eventually leading to multiple organ failure. It is often diagnosed too late when the cardiovascular system has already collapsed (septic shock) increasing mortality risk. Following an NC3R Maths in Medicine workshop, I have been working with a mathematician to maximise the use of blood pressure data obtained by radiotelemetry. By plotting the blood pressure waveform data in 3D space (attractor reconstruction) we are able to extract more information from the signal beyond systolic, diastolic, pulse pressures and heart rate. This quantification of the waveform shape, rather than just the maximum, minimum and rate have enabled us to detect more subtle cardiovascular changes in the earliest stages of sepsis. The measures have reduced variability and we envisage that they may not only enable sepsis experiments to be terminated at an earlier time point (refinement) but that the reduced variability in the output measures means we will necessarily use fewer animals (reduction).

RED.2 RIGOUR, RIGOUR, RIGOUR... PRACTICAL APPROACHES TO IMPROVING THE HARM-BENEFIT RATIO IN ANIMAL RESEARCH

THURSDAY 30 JUNE 2016 **14:30**

MALCOLM MACLEOD (UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

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The potential benefits which might accrue from animal research have in the past been constrained by problems with the design, conduct, analysis and reporting of such studies. Some of these problems (randomisation, blinding) are reasonably straightforward to fix, and the 3Rs community have been at the forefront of efforts to promulgate solutions. Ironically, the problem of underpowering of animal experiments is probably due at least in part to one of the 'R's.

Opportunities to reduce harms include substitution with experimental designs of lower Severity, even if the statistical characteristics of these designs mean that more animals are required. However, the statistical performance (ie the number of animals required to show a biologically important effect) of different designs has not to date been adequately described to allow such judgements to be made.

I will propose a tiered strategy for increasing benefits and reducing harms. While these approaches may at first sight appear burdensome, and difficult to enable even in a pre-competitive commercial environment, there are simple measures which could lead to substantial improvements. Ethical imperatives relating both to the use of animals in research and to the exposure of human study subjects to potential harms dictate that as a community we need to do our best to increase value and reduce waste in research involving animals.

RED.3 REDUCE WITH CAUTION: SMALL SAMPLES BRING FICKLE P VALUES AND BLOATED EFFECT SIZES

THURSDAY 30 JUNE 2016 **15:00**

LEWIS G HALSEY (UNIVERSITY OF ROEHAMPTON, UNITED KINGDOM)

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Where live animal models are essential, a key aim of the Reduction principle of the 3Rs is to minimise the number of animals in studies while ensuring experimental designs remain 'robust'. Given that the most common method of statistical analysis is null hypothesis significance testing, such robustness often focuses on designing experiments to have fairly high statistical power. By increasing the expected effect size between experimental conditions, reducing the measurement noise within conditions, and collecting repeated measures where possible, N can be reduced while keeping statistical power high. However, recent work by colleagues and myself demonstrates that unless the statistical power of a study is very high indeed (>90%), the results of that study tend to be inaccurate and, where deemed 'statistically significant', usually overstated (Halsey et al. 2015). We used simple models to demonstrate that the p value often varies dramatically when a study is repeated; p is fickle and therefore a poor measure of the strength of an experimental result. Furthermore, when p happens to be statistically significant, the sample effect size tends to be an exaggeration of reality, which not only misinforms us about the processes we are studying but also encourages under powering of subsequent studies. The fickleness of p could well explain why there is so much present concern and soul searching about the lack of repeatability in science. Irreproducible and inaccurate science should be minimised for many reasons including ethical ones, especially when animal models are involved.

RED.4 RELEVANCE IS THE 4TH R IN ANIMAL EXPERIMENTS

THURSDAY 30 JUNE 2016 **16:00**

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While there are many reasons to conduct research using animals, the most common one that is publically discussed is the development of treatments for human benefit. Yet many experiments in the biomedical field, particularly those using experimental rodents, fail to translate beneficial effects seen in the animals into successful treatments in man.

Careful review of such translational failures reveal many underlying issues that contribute to this problem. Animal models of specific conditions may be poorly chosen or poorly understood. The outcome measures are commonly not pre-specified and multiple assays may be used until a significant finding is achieved. A statistically significant but small effect in a mouse model is highly unlikely to translate into a better effect in man. Experimental design can be poor with inappropriate statistical analysis. Drug doses and routes of administration are often used that may not be possible in man.

Examples of the above will be presented from the neuromuscular field. The use of well validated outcome measures, appropriate choice of model, good experimental design and dosing from human equivalents rather than for the greatest effect will help to ensure that such biomedical research is relevant to translation to effective human treatments.

RED.5 NC3Rs RESOURCES TO IMPROVE THE DESIGN AND REPORTING OF ANIMAL RESEARCH

THURSDAY 30 JUNE 2016 **16:30**

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The reproducibility of biomedical research using animals has come under scrutiny in recent years, and quality standards in the design, analysis and reporting of *in vivo* research have been flagged as concerns. The NC3Rs has been working in this area over the last ten years and led the development of two key resources to support researchers. The ARRIVE guidelines summarise the minimum information necessary to describe a study and make recommendations on the reporting of the study design, experimental procedures, animal characteristics, housing and husbandry, and statistical analysis. The Experimental Design Assistant is a free online resource, which helps researchers design animal experiments, generating an explicit representation of the experimental plan, as well as providing feedback and dedicated support for randomisation, blinding and sample size calculation. The objective of these resources is to maximise the output of research using animals. Wide dissemination and uptake are essential to ensure the science emerging from animal research is fully exploited.

RED.6 THE UNIVERSALITY OF THE 3R'S OF ANIMAL ETHICS ACROSS A DIVERSE AND GLOBAL RESEARCH COMMUNITY

THURSDAY 30 JUNE 2016 **17:00**

CRAIG E. FRANKLIN (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA)

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This presentation will examine the question: 'How do we achieve consistency with implementing the 3Rs (reduction, refinement and replacement) across a diverse and global research community?' Although the intent is for the 3Rs of animal ethics to be ubiquitous, the 3Rs and ethical standards can mean different things to different people, organisations, states and countries. Ethical standards and the 3Rs can be interpreted differently depending on the type and field of research (e.g. biomedical vs ecological vs conservation), the questions asked, the legislation that is enacted, and the cultural and societal mores that exist. As the biological research world is becoming more and more collaborative and expansive, achieving consistency in the implementation of the 3Rs and maintaining uniformly high ethical standards are the challenges we must consider. Who are and should be the 'gate-keepers' in ensuring that the 3Rs are adhered to, and what are the criteria that should be applied.

EDUC.1 PIONEERING BETTER SCIENCE THROUGH THE 3RS: AN INTRODUCTION TO THE NC3Rs

FRIDAY 1 JULY 2016 **09:15**

MARK J PRESCOTT (NC3Rs, UNITED KINGDOM)

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The UK's National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) is an independent, scientific organization established by government in 2004 to lead the discovery and application of new technologies and methods that minimize the use of animals in research and improve animal welfare (the 3Rs). We take a science-led, collaborative approach to advancing the 3Rs, working with individuals and organisations from across the life sciences sector, nationally and internationally, including universities, the pharmaceutical, chemical and consumer products industries, other research funders and regulatory authorities. Our strategy to improve science and business through application of the 3Rs includes: i) funding basic research, training and career development; ii) supporting open innovation and commercialisation of 3Rs technologies; and iii) bringing scientists together to generate an evidence base to support changes in policy, practice and regulations. In addition to 3Rs impacts, the science we fund is providing knowledge to improve understanding of human diseases, develop efficacious and safe medicines, and protect the environment. Alongside our role as the UK's main funder of 3Rs research and innovation, with over £70 million committed to-date, and our international reputation for cross-sector and cross-discipline data sharing, led by our small in-house team of postdoctoral researchers, we also

assist scientists to implement the 3Rs by providing a range of online information resources, publications and scientific events. This presentation will provide an overview of our activities, outline our current funding opportunities, and highlight some of our key information resources relevant to SEB members.

EDUC.2 BIOTECHNOLOGY AND BIOLOGICAL SCIENCES RESEARCH COUNCIL – BBSRC

FRIDAY 1 JULY 2016 **09:35**

LYDIA DARRAGH (BBSRC, UNITED KINGDOM)

LYDIA.DARRAGH@BBSRC.AC.UK

Funded by Government, BBSRC invested over £509M in world-class bioscience research and training in universities and strategically funded institutes in 2014-15. Our aim is to further scientific knowledge, to promote economic growth, wealth and job creation and to improve quality of life in the UK and beyond. BBSRC promotes animal welfare and responsible use of animals in bioscience research. In order to carry out animal research, BBSRC-funded researchers must demonstrate that they have appropriately considered the '3Rs' in the design of their experiments. Responsible use of animals and appropriate licensing and ethical approvals are conditions of funding.

BBSRC is one of the funders of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), which provides a UK focus for the promotion, development and implementation of the 3Rs in animal research and testing.

BBSRC also funds 3Rs research directly, investing £3.1M in the area during 2014-15. We encourage researchers to look for opportunities to improve animal welfare and/or integrate the 3Rs principles within their work, even where it not the main research focus. Our ongoing strategic priorities in 'The replacement, refinement and reduction (3Rs) in research using animals' and 'Welfare of managed animals' support this approach.

BBSRC delivers funding in these strategic priority areas through Responsive Mode, which has three open calls per year. The January closing date for Responsive Mode hosts an Annual Focus in Animal Welfare, where both welfare and 3Rs research proposals are particularly encouraged.

For more information about BBSRC, see: www.bbsrc.ac.uk.

EDUC.3 WHAT IS LASA?

FRIDAY 1 JULY 2016 **09:55**

CAROLINE CHADWICK (LASA, UNITED KINGDOM)

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LASA - the Laboratory Animal Science Association was founded in 1963 and celebrated its 50 year anniversary in 2013. LASA's aims whose aims are to provide the research community with the highest standards of leadership in the science and welfare which underpins animal research, and in the design and conduct of experiments using animals.

LASA is a charitable organisation and is run by an elected council whose members are all volunteers, serving normally a 3 year term. The expertise on council is widespread and tries to cover across the breath of LASA's activities. Working in partnership with other organisations and learned societies is one of the strengths of the organisation.

Supporting council there are a series of specialist sections which produce many of the societies outputs in the form of published guidance and specialist meetings for the membership. These sections include animal science, education and training, care and welfare, the 3Rs, home office liaison training and information and Establishment Licence holders group.

Membership of LASA covers a wide range of individuals - scientists, veterinarians, animal facility managers and technologists, named persons, animal technologists and representatives from welfare organisations. It is this broad family that makes LASA such an effective representation of the animal science community.

EDUC.4 THE IMPORTANCE OF OPENNESS ABOUT ANIMAL RESEARCH

FRIDAY 1 JULY 2016 **10:15**

WENDY JARRETT (UAR, UNITED KINGDOM)

WJARRETT@UAR.ORG.UK

In 2014, 72 organisations signed up to The Concordat on Openness on Animal Research in the UK. The Concordat currently has 100 signatory organisations and has led to a step-change in the availability of information about animal research in the public domain. Wendy Jarrett will explain the background to the Concordat, how it was developed and some of the results from this work.

EDUC.5 ENGAGING WITH ETHICAL REVIEW WORKSHOP

FRIDAY 1 JULY 2016 **11:10**

PENNY HAWKINS (RSCPA, UNITED KINGDOM)

PENNY.HAWKINS@RSPCA.ORG.UK

Good communication and understanding are essential between scientists and their local Ethical Review Bodies (ERBs), whether these are Animal Welfare Bodies (AWBs), Animal Welfare and Ethical Review Bodies (AWERBs) or Animal Care and Use Committees (ACUCs). All of these institutional committees, or processes, should bring together members with different expertise and perspectives to apply local values when considering whether and how animals are used. The tasks of local ERBs vary between different countries, and may include considering specific ethical and welfare questions arising in relation to individual projects or the facility's wider policies and practice; providing a forum for discussion on matters relating to animal welfare, care and use; and advising on the application of the Three Rs. Ethical review can also include harm-benefit analyses of proposed projects involving animals, and monitoring the development and outcome of projects, including the effect on the animals used and whether there are further opportunities to implement the Three Rs. Good practice for ERBs includes ensuring that scientists are engaged with, and contribute towards achieving, all of the body's tasks - which will benefit both science and animal welfare at the facility. The workshop will include short talks from a regulator, veterinarian, scientist and lay member on the process of ethical review from their viewpoints, providing examples of good practice from local ERBs. Participants will be able to discuss their own engagement with local Ethical Review Bodies and we will identify tips and action points to help ensure effective communication and constructive interactions.

EDUC.6 THE THREE Rs: DEVELOPING AN ETHICAL FRAMEWORK

FRIDAY 1 JULY 2016 **13:30**

JOHN A BRYANT (UNIVERSITY OF EXETER, UNITED KINGDOM)

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How we work with the three Rs in animal research is at its heart an ethical decision. In teaching our students, it is not enough just to present them with three principles, even if we use extensive examples. It is important that they understand how we arrive at ethical decisions and the factors that influence our ethical decision making. Different 'ethical frameworks' may lead to different final decisions. Not only will this understanding help our students to make decisions about animal research and other bioethical questions but it will also aid their more general critical thinking.

EDUC.7 THE USE OF MULTIMEDIA IN BIOETHICS EDUCATION

FRIDAY 1 JULY 2016 **14:10**

DR CHRIS WILLMOTT (UNIVERSITY OF LEICESTER, UNITED KINGDOM)

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All Biological Sciences courses in the UK are expected to offer students the opportunity to reflect on the moral and ethical questions raised by their study discipline. This presentation will look at a variety of ways in which multimedia can be used to promote student engagement with bioethical issues, including the reduction, refinement and replacement of animals in research.

EDUC.8 IMPLEMENTING 3R's: TRANSFERRING STUDENT LEARNING FROM THE CLASSROOM TO LAB AND FIELD

FRIDAY 1 JULY 2016 **14:50**

TERESA G. VALENCAK (UNIVERSITY OF VETERINARY MEDICINE, AUSTRIA)

@ TERESA.VALENCAK@VETMEDUNI.AC.AT

Will this animal bite me? How gently do I have to hold the animal or will I get an infection after it has bitten me? What is my influence on refinement under these circumstances? These and even more questions arise in early career students and researchers when starting to do animal work. Further issues may arise when the 3R's is implemented in the field. Not only does this situation demand a great deal of perseverance and concentration, but the animal's tameness and behaviour will be totally different from that of laboratory animals. Although students are already familiar with the 3R's, after Russel and Burch (1959), many of them do not make the connection with its implementation in practical work. Also, depending on their background, including factors such as cultural differences and whether or not they are familiar with pets and how to handle them, students may need support in using the appropriate movements, and having the right attitude and thoughtfulness. In my talk I will discuss the importance of knowing an animal's social behaviour, its preference for solitary or group living and automatizing handling as a way to optimally refine, reduce and ultimately replace animal experimentation. Furthermore, I will review my experience of potential cultural differences and differences in attitudes towards animal work originating in maturity and personal development.

POSTER ABSTRACTS WEDNESDAY 29 JUNE 2016

REF.12 LOCAL ANAESTHETIC? SYSTEMIC EFFECTS OF SUBCUTANEOUS LIDOCAINE IN THE AMERICAN BULLFROG – *LITHOBATES CATEBEIANUS*

WEDNESDAY 29 JUNE 2016

CATHERINE J A WILLIAMS (AARHUS UNIVERSITY, DENMARK), AAGE K O ALSTRUP (AARHUS UNIVERSITY HOSPITAL, DENMARK), MADS F BERTELSEN (CENTRE FOR ZOO AND WILD ANIMAL HEALTH COPENHAGEN ZOO, DENMARK), CLEO A C LEITE (FEDERAL UNIVERSITY OF SÃO CARLOS, BRAZIL), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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Sodium channel blockers, such as lidocaine, are commonly used local anaesthetics; preventing processing of noxious stimuli (nociception), but their effects on the central nervous system and heart preclude their use at higher doses to induce general anaesthesia in mammals. Here we investigate the effects of subcutaneous injection of lidocaine (5 or 50 mg kg^{-1}) in bullfrogs (*Lithobates catebeianus*) on mentation, reflexes, gular respiration and heart rate (handled group, n=10) or blood pressure and heart rate via an arterial catheter (n=6). 5 mg kg^{-1} lidocaine did not affect alertness, reflexes, or heart rate in the handled group within an hour of injection, but was associated with a reduction in gular respiratory rate (from 99 ± 7 to 77 ± 7 breaths min^{-1}). The higher dose of lidocaine caused further reduction in respiratory rate, no significant change in handled heart rate, but led to heavy sedation, a progressive loss of righting reflex (complete loss by 50 min), palpebral reflex (n=8 loss at 70 min), and contralateral toe pinch withdrawal (complete loss by 70 min). Reflexes were regained over 4 h. Interestingly, systemic anaesthetic effects were not associated with anti-nociception, as a forceps pinch test at the site of injection provoked movement at the height of the systemic effect (70 min). Amphibians are routinely subject to general anaesthesia via exposure to sodium channel blockers such as MS222 or benzocaine, however caution should be exercised when using injectable lidocaine in amphibians, as it appears to dose dependently induce sedation, without necessarily preventing nociception.

REF.13 A NOVEL HOME CAGE MONITORING SYSTEM FOR MULTIPLY HOUSED MICE

WEDNESDAY 29 JUNE 2016

RASNEER BAINS (MRC HARWELL, UNITED KINGDOM), DUNCAN SNEDDON (MRC HARWELL, UNITED KINGDOM), HEATHER CATER (MRC HARWELL, UNITED KINGDOM), ABRAHAM ACEVEDO (MRC HARWELL, UNITED KINGDOM), PATRICK NOLAN (MRC HARWELL, UNITED KINGDOM), SARA WELLS (MRC HARWELL, UNITED KINGDOM

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Many central nervous system disorders and neurodegenerative diseases are investigated using mouse models. Most experimental designs involve removing mice from their homecage and placing them into novel environments to undergo a battery of tests, often conducted for short periods in social isolation. However, human manifestations of such disorders are often progressive and have a large social element. Here we introduce an automated, minimally invasive system for mice to assess disease progression, animal welfare and investigate social behaviours in the homecage environment. MRC Harwell is the sponsor for 'CRACK-IT' initiative by the NC3R 'Rodent Little Brother'. The Home Cage Analysis system, devised by Actual Analytics, Edinburgh, is built around a standard Individually Ventilated Cage and comprises a radio-frequency identification reader baseplate as well as an infrared camera and a computer. With the baseplate and the

camera, we are able to record both activity data, via the tracking of the individual microchips, alongside videos which enables detailed analysis of different behaviours. The current set up has been optimised to three mice in a cage. The unique combination of video and spatial data provides a much richer set of features for analysis and can be achieved through a minimally invasive procedure. In addition the system provides a tool to identify earlier time points for humane intervention for models of neurodegeneration, especially where these have been difficult to detect from cage side assessments. The potential impact for this project on the future direction of welfare and behavioural testing is significant and far-reaching.

REF.14 INVESTIGATING THE TEMPORAL RESPONSE TO A UCMS PROCEDURE AND IDENTIFYING HIPPOCAMPAL STRESS MARKERS IN MICE

WEDNESDAY 29 JUNE 2016

GRACE C LAWS (INSTITUTE OF NEUROSCIENCE NEWCASTLE UNIVERSITY, UNITED KINGDOM), JASMINE CLARKSON (INSTITUTE OF NEUROSCIENCE NEWCASTLE UNIVERSITY, UNITED KINGDOM), JONATHAN COXHEAD (INSTITUTE OF GENETIC MEDICINE NEWCASTLE UNIVERSITY, UNITED KINGDOM), JOHNNY ROUGHAN (INSTITUTE OF NEUROSCIENCE NEWCASTLE UNIVERSITY, UNITED KINGDOM), TIM BOSWELL (SCHOOL OF BIOLOGY NEWCASTLE UNIVERSITY, UNITED KINGDOM), TOM SMULDERS (INSTITUTE OF NEUROSCIENCE NEWCASTLE UNIVERSITY, UNITED KINGDOM)

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Measuring physiological proxies of stress within mice models of chronic disease can allow comparison of model severity to subsequently guide disease model choice. In response to positive stress and distress, the HPA axis is activated. Therefore, stressor valence cannot be distinguished by direct markers of HPA axis activation. Adult hippocampal neurogenesis, however, is a stress sensitive proxy of affective state that is decreased following distress and increased following eustress. The aim of this project was to identify potential stress markers within the mouse hippocampus and to study behavioural and physiological responses to chronic stress across a time-course of unpredictable chronic mild stress (UCMS). C57BL/6 mice underwent exposure to UCMS (n=72) or were housed in standard housing conditions (n=72) for up to ten weeks. Mice in the UCMS condition were administered various stressors in a random nature. At baseline and every two weeks thereafter, mice in both groups (n=24) were sacrificed following ten minutes exploration in an open field. Brain and hippocampal weights were measured at each time point and spleen and adrenal weights were measured at baseline and ten weeks. Hippocampal samples were analysed by RNA sequencing after study cessation from mice in both groups (n=20). This poster will present findings from RNA sequencing, open field behaviour, and organ weight measurements across the time-course. In addition to identifying stress markers, the results aim to allow an understanding of the temporal dynamics of the stress response. Future application intends to quantify stress experienced in comparative mice models of chronic disease.

REF.15 REFINEMENTS IN THERMAL AND MECHANICAL NOCICEPTIVE THRESHOLD TESTING IN MICE

WEDNESDAY 29 JUNE 2016

POLLY M TAYLOR (TOPCAT METROLOGY LTD, UNITED KINGDOM), MICHAEL J DIXON (TOPCAT METROLOGY LTD, UNITED KINGDOM), JENNIFER R DEUIS (UNIVERSITY OF QUEENSLAND, AUSTRALIA), IRINA VETTER (UNIVERSITY OF QUEENSLAND, AUSTRALIA)

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Mechanical and thermal nociceptive threshold testing (NTT) in mice are widely used for pain studies. Hargreaves systems are commonly used for thermal testing and von Frey filaments (vF) for mechanical trials. Each vF data point requires several stimuli producing mathematically derived non-parametric thresholds. Hargreaves testing uses a ramped stimulus in a dedicated environment. We evaluated methods that allow thermal and mechanical NTT to be carried out in the same cage with fewer stimuli applied. Adult male C57BL/6J mice (6-8 weeks, n=12) were tested after sensitisation with carageenan, capsaicin or burn injury and after oxycodone treatment. Sequential thermal and mechanical tests were undertaken after 5 minutes acclimatisation in purpose-built cages. Thermal testing on the hind paw (plantar surface) used a rounded 2.5 mm diameter metal

probe starting at 37°C, heating on contact at 2.5°C/sec. The threshold temperature was automatically recorded. Mechanical NTT was performed with a soft-transducer electronic vF (force range 0-7g) at the same site. Thermal NTT quantified allodynia (untreated and treated respectively) after carrageenan (50.3±0.6; 43.1±1.0°C), capsaicin (49.7±0.6; 44.8±1.2°C) and burn injury (50.8±0.5; 43.2±0.6°C) giving comparable results to the Hargreaves test. Opioid mediated analgesia was also detected. Mechanical allodynia was detected after carageenan (3.4±0.2; 1.4±0.1 gf) and burn injury (3.9±0.7; 1.1±0.2 gf). Thermal and mechanical NTT quantified allodynia and analgesia as well as the Hargreaves system without moving mice for the second modality. Refinements over vF and Hargreaves systems include reduced stress from changing environments, shorter acclimatisation time and fewer mechanical stimuli per data point.

REF.16 BEHAVIOURAL AND PHYSIOLOGICAL MEASURES OF STRESS TO OPTIMISE THE WELFARE AND HENCE SCIENTIFIC POTENTIAL OF LAKE ZACAPU SALAMANDER (*AMBYSTOMA ANDERSONI*)

WEDNESDAY 29 JUNE 2016

CHRIS J EMMANS (UNIVERSITY OF CHESTER, UNITED KINGDOM), ANDREW M HOLMES (UNIVERSITY OF CHESTER, UNITED KINGDOM), ROBERT COLEMAN (UNIVERSITY OF CHESTER, UNITED KINGDOM), CHARLOTTE A HOSIE (UNIVERSITY OF CHESTER, UNITED KINGDOM), TESSA E SMITH (UNIVERSITY OF CHESTER, UNITED KINGDOM)

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Ensuring excellent welfare in captivity is fundamental to having physiologically and psychologically healthy animals to serve as scientifically valid and robust research subjects. The Lake Zacapu salamander (*Ambystoma andersoni*) is critically endangered - 7th on ZSL's EDGE top 100 (Evolutionarily Distinct, Globally Endangered) - and has highly distinctive features, including neoteny. As a result it is of key interest to evolutionary and amphibian biologists. Despite large numbers in captivity the welfare of amphibians has received less attention than other taxa; and research into amphibian welfare is limited and has frequently used invasive measures (e.g. cardiac puncture) which confounds scientific results. Here, novel non-invasive physiological and behavioural bioassays were developed and validated for the first time to quantify stress in *A. andersoni*. Measures of water-borne corticosterone release rate, respiration rate and behaviour were used to assess the impact of a routine health check on this species. *A. andersoni* displayed significantly higher respiration rates as well as a tendency to be less active following the health check (compared to a control condition). There was also a non-significant trend for higher water-borne corticosterone release rates following the health check compared to control conditions. The development of these physiological and behavioural measures of amphibian welfare allows for the systematic optimisation and refinement of the captive environment of *A. andersoni*. Higher quality data (produced by animals with good welfare) also reduces the numbers of animals required for statistically valid experimentation in the laboratory setting hence impacting directly on the 3Rs (animal reduction).

REF.17 NON-INVASIVE ASSESSMENT OF A RANGE OF ENRICHMENTS ON THE WELFARE OF LABORATORY-HOUSED *XENOPUS LAEVIS*

WEDNESDAY 29 JUNE 2016

ANDREW M HOLMES (UNIVERSITY OF CHESTER, UNITED KINGDOM), CHRISTOPHER J EMMANS (UNIVERSITY OF CHESTER, UNITED KINGDOM), ROBERT COLEMAN (UNIVERSITY OF CHESTER, UNITED KINGDOM), TESSA E SMITH (UNIVERSITY OF CHESTER, UNITED KINGDOM), CHARLOTTE A HOSIE (UNIVERSITY OF CHESTER, UNITED KINGDOM)

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The housing environment of captive animals can profoundly influence their welfare and may impact upon the purpose of their captivity (e.g. research, production or conservation). In laboratory species poor welfare can reduce the reliability and repeatability of scientific results. Refinement of the housing requirements of laboratory species is therefore crucial for high quality research and may ultimately result in a reduction in numbers of laboratory animals required. Much work has explored and identified suitable captive housing

conditions for most model species, but not amphibians. The African clawed frog (*Xenopus laevis*) is a common model organism in biomedical research but current husbandry guidelines lack supporting quantitative evidence. Establishing optimal housing conditions for *X. laevis* is essential both for improving welfare and maximising the quality of scientific research using this species. The quantification of adrenal steroids in the tank water for aquatic amphibians presents a valuable way to assess welfare since it involves minimal disturbance and consequently results are not affected by sampling methods. We have immunologically and biologically validated an immunoassay to quantify water-borne corticosterone for *X. laevis*. We have then used this assay in combination with behavioural observations to investigate a variety of physical and social enrichment housing parameters. For example different background tank colours significantly alter both corticosterone and behaviour. These results will help in refining the captive housing of this common model species and go towards establishing optimal husbandry protocols.

REF.18 ALFAXALONE ANAESTHESIA IN THE BALL PYTHON *(PYTHON REGIUS)*

WEDNESDAY 29 JUNE 2016

LAUREN E JAMES (AARHUS UNIVERSITY, DENMARK), CATHERINE JA WILLIAMS (AARHUS UNIVERSITY, DENMARK), MADS F BERTELSEN (COPENHAGEN ZOO, DENMARK), TOBIAS WANG (AARHUS UNIVERSITY, DENMARK)

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The use of an induction agent is recommended when considering reptile anaesthetic protocols to enable rapid and safe intubation with minimal stress caused to the animal. Inhalant agents can give variable induction times; therefore the use of injectable agents is recommended. Injectable anaesthesia is well-established in reptiles and facilitates induction of surgical anaesthesia and sedation for handling of agitated or potentially dangerous animals. However, vascular access in snakes may be challenging, so it is useful to have access to induction agents that provide anaesthesia when delivered intramuscularly. Alfaxalone is a synthetic neuroactive steroid that produces sedation and muscle relaxation and can be administered safely either intravascularly or intramuscularly. Successful anaesthetic induction upon intramuscular injection has been reported in several reptile species, however there is little information on the use of alfaxalone in snakes. Six ball pythons (*Python regius*) were each administered three doses (10, 20 and 30 mg/kg) of alfaxalone in a randomised cross-over design, and the quality of anaesthesia was assessed. The time to loss of righting reflex and muscle tone, alongside respiration rate and the ability to intubate with an endotracheal tube were recorded. We also assessed the anaesthetic's potential for the provision of analgesia using mechanical noxious stimulation with a pinch to the tail tip using forceps. Our aim was to determine an appropriate dose of alfaxalone to allow endotracheal intubation, which would facilitate maintenance of surgical anaesthesia with other agents, for example inhalant anaesthetics.

REF.19 REFINING SEVERITY LIMITS WITH A REFINED HEALTH MONITORING SYSTEM IN ZEBRAFISH

WEDNESDAY 29 JUNE 2016

KAREN DUNFORD (UNIVERSITY COLLEGE LONDON, UNITED KINGDOM), CAROLE WILSON (UNIVERSITY COLLEGE LONDON, UNITED KINGDOM)

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Health monitoring is imperative to maintaining high quality welfare and husbandry practices. The Animals (Scientific Procedures) Act 1986 and the 3Rs dictate the prevention and minimisation of pain, suffering, and distress experienced by the animals. Any clinical signs of disease or adverse effects from both husbandry and procedures, including genetic alteration, should be recorded and/or reported, not only as a legal requirement, but also as key information that can then be used to identify patterns and issues that should be rectified. At the UCL Zebrafish Facility, using our refined health monitioring protocol, we have standardised how mortality and disease are recorded, allowing 15 years of health records to be analysed in order to identify patterns and issues for specific genetic strains. Some patterns we have thus far identified relate to age and development of specific abnormalities and diseases. This knowledge gives the fish facility users the ability to predict patterns and therefore the opportunity to lower pain, suffering, distress and severity limits in different strains of fish;

this also allows facility staff to provide additional information about strains to PiLs, therefore providing a more powerful tool for strain choice and colony management. With these tools at our disposal, we can now refine severity limits, and reduce numbers of animals used. This system falls under the 3Rs because: it is a refinement of health monitoring protocols; it has improved and standardised training; improved and standardised recorded deaths and illnesses; and decreased the number of fish reaching protocol severity limits.

REF.20 MEASURING MURINE BLOOD PRESSURE: A STUDY TO UNDERSTAND THE PARAMETERS THAT LEAD TO BEST PRACTICE

WEDNESDAY 29 JUNE 2016

ELENA WILDE (KING'S COLLEGE LONDON, UNITED KINGDOM), AISHA AUBDOOL (KING'S COLLEGE LONDON, UNITED KINGDOM), PRATISH THAKORE (KING'S COLLEGE LONDON, UNITED KINGDOM), SUSAN BRAIN (KING'S COLLEGE LONDON, UNITED KINGDOM)

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Accurate blood pressure measurement is crucial for research. There are two techniques to measure blood pressure (BP) in conscious mice: telemetry and tail-cuff plethysmography. Radio-telemetry, involving major recovery surgery, allows continuous measurement in un-restrained animals. Tail-cuff plethysmography is non-invasive and less expensive, but involves handling, restraint and warming the animals. We have examined how these parameters contribute to the end results. We inserted ratio-telemetry probes into the mice and, after recovery, compared the results from telemetry and tail-cuff. We designed protocols to assess the 3 different handling techniques, restraint, heating and cuff inflations/deflations. All handling interventions caused similar significant increases in BP. Restraint, heating and tail-cuff recording process did not further augment the BP, which was maintained throughout the tail-cuff procedure, as measured by telemetry. The tail cuff measurements were significantly lower than the simultaneous telemetry readings (systolic BP 113.113.8 and 153.1±3.5 mmHg respectively, n=8, $p<0.05$). We noted that baseline blood pressure measured by tail cuff was similar to that for telemetry in undisturbed mice. So we compared tail-cuff measurements with non-simultaneous telemetry recordings, obtained before any handling took place. These results were similar between tail-cuff and telemetry, even in a model of hypertension. Our results support the fact that tail-cuff techniques can be used to monitor BP in experimental protocols. However, handling and restraint do affect the mouse, such that telemetry readings that reflect central BP are substantially different, in terms of magnitude than those measured by tail-cuff. The underlying mechanisms are unclear.

REF.21 IMPROVING THE WELFARE OF RAINBOW TROUT DURING ANAESTHESIA

WEDNESDAY 29 JUNE 2016

LYNNE U SNEDDON (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), KIERAN C POUNDER (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM), TOM G POTTINGER (CEH LANCASTER, UNITED KINGDOM)

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Anaesthetics are commonly administered to fish in in research and veterinary contexts. Anaesthesia causes sedation and loss of consciousness and may reduce the stress and/or pain associated with handling and invasive procedures thereby improving welfare. However, these drugs may cause an aversive response. Therefore it is vital to have evidence for the behavioural and physiological responses to a range of drugs. This study sought to establish which of several commonly used anaesthetics exhibited fewest adverse effects in rainbow trout, a laboratory model salmonid, when used for sedation or euthanasia by exploring their effects on aversion behaviour and stress physiology. Five widely used anaesthetics were investigated: MS-222, benzocaine, 2-phenoxyethanol, etomidate and eugenol. The anaesthetics were administered via immersion and fish were: 1) euthanised with anaesthetic; or 2) allowed to recover from deep plane anaesthesia; or 3) subjected to a conditioned place avoidance paradigm. Behaviour, opercular beat rate and plasma cortisol concentrations and cortisol release rates to water were quantified to investigate differences between the five drugs under the three conditions tested, when fish were anaesthetised in a preferred place. Our findings suggests the widely-used and recommended anaesthetic MS-222 was relatively more stressful for rainbow trout and that 2-phenoxyethanol may be a more advisable alternative

due to quicker induction, reduced behavioural response during anaesthesia and lower post-anaesthesia plasma cortisol levels. It is crucial for the welfare of fish that the use of anaesthetics is as humane as possible and thus these findings have important implications for the welfare of captive fish.

REF.22 FISH ON THE MOVE: ASSESSING WELFARE DURING TRANSPORT

WEDNESDAY 29 JUNE 2016

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Few studies highlight the impact of transportation methods on the welfare of farmed fish, particularly salmonids. Even more limited, are studies into the effects of time in transportation and stocking density within transport containers. Transportation of fish from farms to research premises is often undertaken in high densities and in single containers, which can make interpretations on stress levels and changes in the animal's physiology and behaviour difficult. Ensuring a high level of care during transport and husbandry is imperative for high quality data output when using animals in scientific studies. Here we report some preliminary findings on the effects of transport time and stocking densities in transport containers on fish stress (cortisol release) and water quality. In-line with the 3Rs, these physiological traits were measured using non-invasive sampling methods from water and mucus samples (Refinement), meaning fish could be used in other research projects (Reduction). It is hoped that the outcomes of this study will initiate some interest within the research community to investigate the effects of transportation on the welfare of a range of farmed species and those used for the aquarium / hobbyists trade. We also aim to establish a knowledge transfer platform with fish producers to discuss potential new, or improvements to current, remediation methods with which to improve fish welfare during transport. We believe this to be a positive approach to improving experimental approaches in studies utilising live fish, where small changes based on our research could make meaningful improvements to fish transport conditions.

REP.12 NEURONAL FUNCTIONS INHIBITED BY NEONICOTINOID INSECTICIDES IN THE AQUATIC MODEL ORGANISM *LYMNAEA STAGNALIS*

WEDNESDAY 29 JUNE 2016

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Neonicotinoids are widely used agrochemicals with high selectivity towards insects compared to vertebrates. The water solubility and persistence of neonicotinoids, however, also suggest the potential exposure of non-target, aquatic animals in the local environment. We used the pond snail *Lymnaea stagnalis* as a model organism for testing insecticide products containing neonicotinoids as their active ingredients (acetamiprid, imidacloprid, thiamethoxam, clothianidin, thiacloprid). In the isolated central nervous system (CNS) an identified cholinergic (VD4-RPeD1) synapse was reversibly inhibited by neonicotinoid insecticides in the bath. Thiacloprid (0.01 mg/ml) blocked almost 90 percent of the postsynaptic potentials, while the less effective thiamethoxam (0.1 mg/ml) reduced the synaptic responses by about 15 percent. Intact specimens of *Lymnaea stagnalis* were also treated by (0.01 - 0.1 mg/ml) neonicotinoids while the feeding and locomotory activity were tested as behavioural endpoints of toxicity assays. After 30 min exposure acetamiprid blocked the sucrose-evoked feeding response while thiacloprid reduced the feeding rate to about 10% of its control value (this effect lasted for 24 hours). Acetamiprid and thiacloprid also reduced the spontaneous locomotion (10-15 %), while imidacloprid proved to be the less effective blocker of both behaviours. We conclude that neonicotinoid insecticides act on the acetylcholine receptors in the CNS of molluscs, while behavioural data suggest additional, probably neuromuscular modulation in the periphery. These data provide

the first results concerning neonicotinoid-related neuronal effects on a mollusc and also confirm that snails provide a suitable model for further studies of the behavioural/neuronal consequences of neonicotinoid intoxication.

REP.13 DEVELOPMENT AND ADAPTATION OF AN *IN VITRO* RAINBOW TROUT GILL MODEL FOR USE AS AN ALTERNATIVE TO LIVE FISH STUDIES

WEDNESDAY 29 JUNE 2016

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The development and validation of reliable *in vitro* methods that offer an alternative to conventional *in vivo* studies is becoming increasingly important. We have recently initiated methods of primary cell culture for different cell types of rainbow trout (liver, gut and gills), and aim to combine these tissues in co-cultures to provide an *in vitro* model with a higher degree of predictivity towards *in vivo* responses. Here we report on work investigating the further development and adaptation of an existing double-seeded gill epithelial model that is grown on a cell culture insert within a microplate well. The model is a humane alternative to *in vivo* studies of gill physiology, toxicity testing, bioaccumulation studies and water quality monitoring. The study aims were to investigate the effect of different culture methods on the gill model viability and to maximise its lifespan and functionality. We found that the time taken to produce the cultures could be reduced by removing blood cells from the gills via perfusion prior to use. Culture assessment via trans-epithelial resistance and scanning electron microscopy revealed that cells did not adhere to membranes with higher porosity, but that time to confluence and response to apical water addition could be improved by supplementing with native serum and by growing under rotational flow. Indeed, gills from larger fish (~400g) could be cultured successfully and the fish provide their own serum for later culture. Future work aims to further characterise this robust model and use within toxicological and physiological research.

REP.14 CHARACTERIZING UPTAKE AND EFFLUX OF PHARMACEUTICALS AND PPCP USING FISH *IN VITRO* MODELS

WEDNESDAY 29 JUNE 2016

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There are currently tens of thousands of fish being used to assess the uptake and bioaccumulation of compounds under legislative requirements. To develop an *in vitro* gill model to replace the numbers of fish used in these tests it will be important to gain a better mechanistic understanding of the uptake process. Uptake of xenobiotics across the gill of fish is complex. Particularly for ionisable xenobiotics where changes in branchial epithelial boundary layer pH could profoundly influence uptake. We aim to improve the understanding of mechanisms governing the uptake and efflux of various compounds, and have set three main aims in order to achieve this. The first is to measure the pH of the fish *in vitro* gill cell culture system (FIGCS) boundary layer, the second is to characterize and localize potential transporter proteins on the gill that play a role in personal care products and pharmaceutical uptake, and thirdly the project aims to assess uptake and excretion of ionisable compounds across FIGCS in differing water chemistries. This information will be used to develop uptake or accumulation models and help to identify those compounds that have the propensity to accumulate in fish and thus may be of environmental concern.

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