Swiss 3Rs Day

60 years of Replacement, Reduction and Refinement of Animal Experimentation

2 September 2019, Hotel Kreuz, Bern

www.swiss3rsday.com

events@swiss3rcc.org

Welcome

On behalf of the organising committee, we warmly welcome you to the Swiss 3RCC 3Rs Day on 2 September 2019, at the occasion of the 60th anniversary of the 3Rs principle described by Russel & Burch in their book *The Principles of Humane Experimental Technique*.

The 3Rs Day is intended to provide up-to-date information on the Replacement, Reduction and Refinement of animal experimentation (the 3Rs principle) through dedicated sessions on each of the 3Rs.

The 3Rs Day represents a unique opportunity for Swiss scientists to interact with peers and learn about progress in the application of the 3Rs principle. At this occasion, the 3RCC will also announce its 2019 Open Call for Projects, through which it funds high-quality 3Rs research projects.

We hope you will enjoy the scientific programme and that you will return to your work inspired by new ideas and keen to work with newly found colleagues to further advance the implementation of the 3Rs principle in Switzerland.

Enjoy the event!

Kathy Riklin
3RCC President

Chantra Eskes
3RCC Director

Scientific & organising committees

Scientific Committee

Chantra Eskes, 3RCC, Bern

Isabelle Desbaillets, 3RCC, Bern

Elena Dolgodilina, ETHZ, Zurich

Paulin Jirkof, University of Zurich

Matthias Lutolf, EPFL, Lausanne

Armand Mensen, 3RCC, Bern

Marjolaine Philit, University of Geneva

Anke Rohlfs, University of Basel

Thomas Singer, Roche, Basel

Hanno Würbel, University of Bern

Organising Committee

Chantal Britt, 3RCC, Bern

Isabelle Desbaillets, 3RCC, Bern

Chantra Eskes, 3RCC, Bern

Armand Mensen, 3RCC, Bern

The official language of the conference is English. No simultaneous translation is provided.

Certificate of attendance

Participants can download their *Certificate of Attendance* on the congress website using their personal login created for registration. This certificate of attendance is not the certificate for continuing education mentioned below and therefore should not be presented to the Swiss veterinarian authorities as such.

Continuing education certificate

The Swiss 3Rs Day has been accredited as one day of continuing education for study directors and experimenters by the Federation of Swiss Cantonal Veterinary Officers (VSKT).

Study directors and experimenters working in Switzerland can obtain the continuing education certificate ONLY if they have signed the list of attendance at the registration desk at the beginning and at the end of the meeting.

If you cannot stay the entire day, please sign out at the registration desk when you leave. If you follow a minimum of 3 hours conference, you will receive a 0.5 day certificate (for this you must follow minimum 3 hours conference).

Following the conference, attendees will be able to download the certificate when they login to the event website.

3RCC Young Scientists Awards

The 3RCC will select one Best Poster presentation and one Best Oral presentation award to young scientists i.e., PhD students and postdocs with no more than five years post-doc experience.

The 3RCC Young Scientist Award represents CHF 300 for each awardee and will be announced during the closing ceremony of the Swiss 3Rs Day.

The awardees will be selected based on the quality of their research, its impact on 3Rs, its benefits compared to existing methodologies and the clarity of their presentation. The authors should be present at the closing ceremony to be eligible for the award.

About the Swiss 3R Competence Centre

The Swiss 3R Competence Centre (3RCC) was founded in March 2018 as a non-profit association including representatives from the major 11 universities and higher education institutions working in life sciences in Switzerland, the Swiss Federal Food Safety and Veterinary Office (FSVO), the Swiss association of the pharmaceutical industry, Interpharma, and the Swiss Animal Protection (SAP). The mission of the 3RCC is to promote the principles of the 3Rs (reduction, refinement and replacement of animal experimentation) in Switzerland and to facilitate their implementation in life sciences, focusing on research, education and communication. More information: https://swiss3rcc.org

Main activities:

- Promote high-quality research and care for animals by subsidising scientific projects of excellence and quality on the 3Rs principle.
- Develop a 3Rs education strategy targeted at different educational programmes.
- Build a network and communication platform, providing up-to-date information on the 3Rs principle and alternative methods to animal experimentation to stakeholders and all those interested.

The 3RCC also monitors progress made in the implementation of the 3Rs principle in Switzerland. It offers its services to authorities, teaching bodies and any other interested parties willing to gain additional information on the 3Rs principle and alternative methods to animal experimentation.

3RCC Funding programme

The Swiss 3R Competence Centre publishes regular calls for funding of high-quality research projects dedicated to the replacement, reduction or refinement of animal experimentation.

The calls for projects are in the form of open calls and targeted calls i.e., addressing specific challenges for the advancement of the 3R principles in Switzerland.

3RCC Open Call 2018: six funded projects

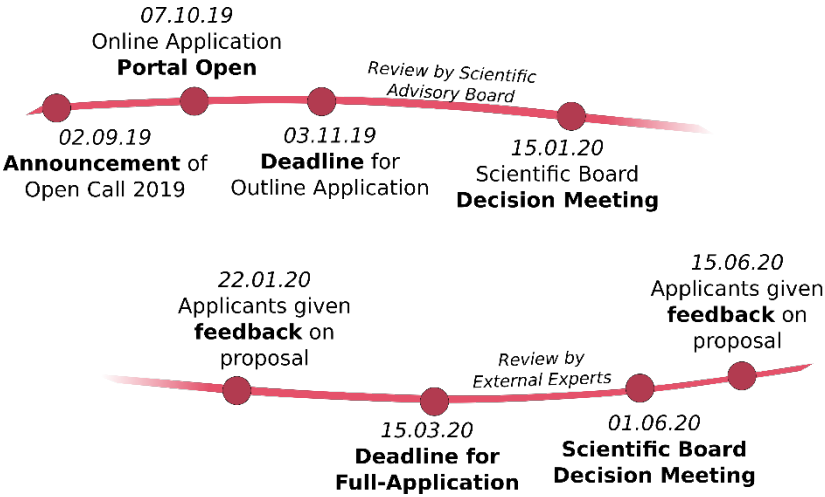
The 3RCC launched its first call for funding in November 2018. It received as many as 54 applications for its first call for projects, with half of the projects focused on replacement, 30% on reduction, and 20% on refinement. The centre selected six projects for funding that aim to replace, reduce and refine animal experiments at Swiss research institutions with CHF 1.2 million. The projects include novel approaches to improve cell cultures and organoids that replace animals used in experiments as well as new strategies for surgery training and breeding with the aim to improve animal welfare and reduce the number of living animals necessary.

You can find a synapsis and more detailed information on the projects on: https://swiss3rcc.org/2019/05/19/funded-projects.

3RCC Open Call 2019

Total funds available: CHF 1,375,000 to fund each of the 3Rs

2 September 2019:	Announcement of Open Call 2019
7 October 2019:	Opening of Call
31 October 2019:	Deadline for outline submissions
22 January 2020:	Applicants notified
15 March 2020:	Deadline for invited full submissions



Scientific Programme

Programme Overview

9.15 – 10.00	***Opening and presentation 3RCC 3Rs Award***	
9.15 - 9.30	Official opening of the 3Rs Day	Kathy Riklin, National Council and president of 3RCC
9.30 – 9.50	Presentation from 3RCC 2019 3Rs Awardees	
9.50 – 10.00	Award ceremony	
10.00 - 11.30	**Session I: Replacement**	Co-chairs: Matthias Lütolf & Thomas Singer
10.00 - 10.25	3Rs and modern safety and discovery technologies	Thomas Singer Roche, Basel
10.25 - 10.50	Improving organoid reproducibility and function through engineered stem cell microenvironments.	Matthias Lütolf EPFL, Lausanne
10.50 – 11.03	Modelling Alzheimer's disease in three-dimensional human neural progenitor cultures.	Laura Suter-Dick FHNW, Muttenz
11.03 – 11.16	Functional microvasculature-on-chip to investigate vascular remolding upon cyclic stretch	Soheila Zeinali ARTOG, Bern Young Scientist
11.16 – 11.30	*In vitro* and *in silico* testing strategies for predicting human liver toxicity from foodborne chemicals	Fabrice Müller ETH Zurich Young Scientist
11.30 – 12.00	*Coffee break & REPLACEMENT poster presentations*	
12.00 - 12.45	**Nonanimal-derived affinity-reagent antibodies**	Chair: Chantra Eskes
	Andreas Plückthun (UZH) and Pierre Cosson (UniGe)	
12.45 - 13.45	*Lunch break & REDUCTION poster presentations*	

13.45 – 15.10	**Session II: Reduction**	Co-chairs: Hanno Würbel & Armand Mensen
13.45 – 14.05	Standardization and the reproducibility paradox in animal research	Hanno Würbel University Bern
14.05 – 14.25	Multi-spectral optoacoustic tomography at the service of the 3Rs	Daniel Razansky UZH & ETH, Zürich
14.25 – 14.45	Avoiding statistical power shortage in a 3R perspective	Romain-Daniel Gosselin CHUV, Lausanne
14.45 - 15.05	AniMatch, an innovative web-based platform to share organs and tissues.	Annemarie Lang, Charité, Berlin, Germany Young Scientist
15.10 – 15.40	**Plenary lecture: Best practice and alternatives in education and training**	Nick Jukes InterNICHE United Kingdom
15.40 – 16.10	*Coffee break & REFINEMENTposter presentations*	
16.10 – 17.45	**Session III: Refinement**	Co-chairs: Paulin Jirkof & Isabelle Desbaillets
16.10 – 16.35	How a culture of care can improve animal welfare	Paulin Jirkof University Zürich
16.35 – 16.55	Development of a sustained-release depot formulation of buprenorphine for pain relief in experimental animals	Viktoria Schreiner University Basel Young Scientist
16.55 – 17.15	Report of the FSVO 3R Symposium – Alternatives to CO_2	Thom Gent University Zürich
17.15 - 17.30	Investigating severity and analgesia in the acute Cerulein-induced pancreatitis mouse model.	Mattea Durst University Zürich Young Scientist
17.30- 17.45	One Europe: The challenge of consistency in severity classification	Anne Zintzsch University Giessen, Germany Young Scientist
17.45 – 18.00	**Closure & awards announcement**	

Swiss 3RCC 3Rs Awardees 2019

During the opening of the Swiss 3Rs Day, the 3RCC will announce its 3Rs Award 2019 granted to two scientists who have significantly contributed to the advancement of Replacement, Reduction, Refinement of animal experimentation (the principle of 3Rs) in the area of life sciences.

Ms Melanie Fischer, Prof. Kristin Schirmer, Swiss Federal Institute of Aquatic Science and Technology (EAWAG)

Lay summary for Swiss 3RCC 3R Award 2019

Thousands of man-made chemicals play important roles in our daily lives. To ensure that their use does not harm human and environmental health, chemicals need to undergo safety testing. To this day, safety testing heavily relies on animal experiments, specifically with fish in the case of environmental hazard assessment. One of the most frequently, but also most severe tests pursued in this regard is the fish acute toxicity test: fish are exposed to chemicals or water samples for four days and death is recorded. Given its frequent and global application, the estimated number of fish sacrificed per year in this way is in the millions.

To replace this type of test, the group of Kristin Schirmer has developed a cell line assay, which reliably predicts fish acute toxicity for a wide range of chemicals and water samples. The assay is based on the gill cell line, RTgill-W1, originating from rainbow trout (Oncorhynchus mykiss), and measures cell survival as indicator for gill integrity in fish. After careful establishment, the assay has been thoroughly tested and validated, first by the Schirmer group and subsequently in an international round-robin study with laboratories from industry and academia. This latter study demonstrated the robustness of the RTgill-W1 cell line assay and its accurate performance when carried out by operators in different laboratory settings. Thanks to these efforts, the International Standardization Organization (ISO) has recently approved the assay as the first international standard based on a fish cell line – rendering it a forerunner in this regard.

Session I. Replacement

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11.30 – 12.00	*Coffee break & poster presentations REPLACEMENT*	

Oral presentations – Replacement session

10h00 – 10h25

3Rs and modern safety and discovery technologies

Thomas Singer, Pharmaceutical Sciences, Pharma Research & Early Development, Hoffman La Roche, Switzerland

This is a time of unprecedented innovation with many remarkable possibilities to pursue the promise of developing transformative medicines for patients. Animal studies have been an important part of defining the efficacy and safety of drugs before their use in humans. At the same time, there are tremendous opportunities to pursue and incorporate replacement methods, based on modern technologies, to reduce the volume of animal testing in drug development. At Roche, our goal is to conduct rigorous science while embracing the 3Rs principles. We are doing this by a growing use of alternative methods and negotiating with Health Authorities for adoption of these new methods as part of regulatory guidelines for modern drug development.

This presentation will highlight the alternative methods and approaches that are being taken to reduce animal testing and improve the transferability of results to humans during drug development. These approaches include the use of innovative *in silico*/computational modeling and simulation, advanced analytics, and human derived cell - based organ-on-a-chip models that minimise the number of experiments and animal studies, while making preclinical drug development programmes more predictive for clinical trials. We are also setting new standards in terms of infrastructure and *in vivo* work environment with the construction of a new Roche *in vivo* research center. This new facility is designed with a focus on both animals and humans, to provide optimised housing for animals and the best facilities for animal caretakers and researchers to conduct their work efficiently and effectively. As a result, we can anticipate an impact on the quality of data and the number of experiments involving animals that are needed for evaluating drugs. Taken together, these innovative technologies and state-of-art infrastructure will help us take a major step forward in the way we develop new drugs, while reducing animal testing, in the future.

10h25 – 10h50

Improving organoid reproducibility and function through engineered stem cell microenvironments

Matthias P. Lütolf, Laboratory of Stem Cell Bioengineering, Institute of Bioengineering, School of Life Sciences and School of Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their *in vitro* counterparts hinders their even wider applicability. In this talk I will discuss some of our ongoing efforts in developing next-generation organoids that are assembled by guiding cell-intrinsic self-patterning through engineered stem cell microenvironments. The improved reproducibility and function of these engineered organoids opens up exciting prospects for drug discovery and regenerative medicine.

10h50 – 11h03

Modelling alzheimer's disease in three-dimensional human neural progenitor cultures

C. Gaiser, A. Weston, L. Suter-Dick

University of Applied Sciences and Arts Northwestern Switzerland, School for Life Sciences, Muttenz, Switzerland

Introduction: Alzheimer's disease (AD) research currently relies on many transgenic models resulting in the widespread use of animals for the study of the disease. Unfortunately, there are a lack of suitable *in vitro* models that recapitulate the key features of AD; namely the accumulation of amyloid beta (Abeta) plaques, the formation of neurofibrillary tangles and neuro-inflammation leading to loss of neurons and synaptic connections.

Methods: in our attempt to replace animal models with regard to the "3Rs" (reduction, replacement and refinement) principles, we took advantage of three-dimensional (3D) cell culture alternative methods to generate a neuronal Matrigel-based *in vitro* model of familial AD (FAD) with mutations in human amyloid precursor protein (APP) and human presenilin 1 (PSEN1).

Results: Our data demonstrate that the generated FAD cell line carries the expected genotypes: APP with both K670N/M671L (Swedish) and V717I (London) mutations and PSEN1 with deleted exon 9. The engineered cells differentiated into neurons and astrocytes, as detected by specific neuronal (MAP2 and Tuj1) and astrocyte (GFAP) markers. Long-term (up to 12 weeks) maintenance of these cells in 3D-cultures led to the development of AD-pathology in *vitro*, defined by increased Abeta-secretion, Abeta-deposition and tau-hyperphosphorylation. The FAD neurons grown in Matrigel-based 3D thin-layers exhibited a higher number of Abeta deposits after 6 weeks of differentiation compared to control cells as well as hyperphosphorylated Tau after 9 weeks of differentiation as evidenced by immunofluorescence studies. An imbalance on the three-repeat (3R) to four-repeat (4R) splicing ratio of Tau was also detected by RT-PCR.

Conclusions: Taken together, our results show that our *in vitro* human FAD model successfully recapitulates both the Abeta and tau pathologies. Such a cellular tool could further help elucidate pathological mechanisms of AD and accelerate drug discovery by identifying new targets and drugs for AD patients.

11h03 – 11h16

Functional microvasculature-on-chip to investigate vascular remolding upon cyclic stretch

S. Zeinali[1], E.K. Thompson[1], T. Geiser[1,2,3], O.T. Guenat[1,3,4]

[1]Organs-on-Chip Technologies Laboratory, ARTORG Center, [2]Department of Biomedical Research, University of Bern, [3]Department of Pulmonary Medicine, [4]Division of General Thoracic Surgery, University Hospital of Bern, Bern, Switzerland

Introduction: Vascular cells can sense variations in mechanical forces and transduce the mechanical signal into a biological response. However, the precise mechanisms of vascular cell mechanotransduction remain a mystery. Current *in vitro* microvasculature models investigating cellular response induced by changes in the mechanical environment are limited to the study of the effect of the shear stress generated by the blood flow. The existing models that incorporate mechanical stretch are usually limited to two-dimensional cell culture platforms, which lack the dimensionality seen by cells. Advanced three-dimensional *in vitro* microvasculature models offer a potential alternative to traditional animal experiments. These platforms are seen as promising tools to address underlying mechanisms of pathophysiological mechanotransduction. In this research we developed a dynamic microvasculature platform to investigate the effect of cyclic stretch on human microvascular remodelling.

Methods: Experiments were performed using a microfluidic platform. The platform was a multi-layered system that consists of a thin polymeric membrane and a fibrin gel layer with an incorporated microvasculature cavity. A perfusable microvasculature was reproduced in the fibrin gel layer by seeding human endothelial cells in a tube formed by a needle that was removed after gelation of the fibrinogen. After formation of an endothelial barrier, the microvasculature was subjected to a cyclic strain. After immunostaining, confocal images of the microvasculature were analysed to examine the microvascular structure and morphology.

Results: Cyclic stretch appears to improve the integrity of the microvasculature, by assisting the formation of tighter vessel walls. Results of the experiments exposed that cell membranes, cell attachment sites, and cytoskeletal network serve as primary mechanosensors because of mediating signal transduction by activating transmembrane receptors of cadherin (cell-to-cell) and integrin (cell-to-substrate) families. The model revealed strong differences in the permeability, morphology and structure of microvessels in either static or dynamic conditions.

Conclusion: This *in vitro* dynamic model of human microvasculature represents a promising approach because it permits to study the effect of physiological and pathological cyclic stretch on isolated human microvessels. This platform provides the possibility to study the mechanotransduction mechanism and the vascular remodeling in greater detail than *in vivo* studies.

11h16 – 11h30

In vitro and *in silico* testing strategies for predicting human liver toxicity from foodborne chemicals

F.A. Müller, M. Stamou, S. Diedrich, S.J. Sturla

Department of Health Sciences and Technology, ETH Zurich, Zürich, Switzerland

The increasing number of food-related chemicals poses a huge challenge in risk assessment. Currently, evaluating toxicological hazards of new chemicals with animal testing approaches are outdated and their ethical and financial limitations are widely acknowledged. At the same time, non-alcoholic fatty liver disease (NAFLD) is a rising global burden affecting around 30% of the adult population. NAFLD encompasses a complex disease spectrum ranging from benign steatosis to non-alcoholic steatohepatitis that can progress to cirrhosis and liver failure. The prevalence of NAFLD and its link to obesity and metabolic disorders suggests that environmental exposures could contribute to NAFLD. Systematic assessments of chemicals as NAFLD risk factors have not been carried out in part due to a lack of validated *in vitro* and *in silico* testing strategies. In order to address this gap, we developed a high content imaging approach for simultaneously quantifying mechanism-based NAFLD markers in metabolically competent human liver cells and extrapolating human oral equivalent doses for molecular responses. Various exposures with known NAFLD-relevant pathophysiology were evaluated. Cells were fluorescently stained for nuclei, lipids, mitochondrial dysfunction and oxidative stress and imaged on a high content imaging system. An algorithm was developed for automated image processing and cell death, lipid droplet number and size, mitochondrial dysfunction and oxidative stress were quantified on a population as well as single cell level. Oral equivalent doses were extrapolated using physiologically based pharmacokinetic modelling along with reverse dosimetry. Chemicals induced a concentration-dependent increase in lipid accumulation, disruption of mitochondrial function and integrity, and increase in oxidative stress, including heterogeneous responses of single cells in the population.

Extrapolated oral equivalent doses for the key events addressed were within biologically relevant exposure ranges for the test chemicals. Future research will aim to establish a predictive model and screen food-related chemicals for their risk to induce NAFLD.

Poster presentations – Replacement session

Poster 8

Modeling pediatric encephalopathies in Drosophila

M. Savitskiy[1], G. Solis[1], V. Katanaev[2]

[1]Department of Cell Physiology and Metabolism, Université de Genève, Geneva, [2]Department of Cell Physiology and Metabolism, Université de Genève, Nyon, Switzerland

G protein-coupled receptors (GPCRs) constitute the biggest receptor family in metazoans and signal through heterotrimeric G proteins, of which the α-subunits provide the signal transduction specificity. The αo-subunit (Gαo) is the major G protein expressed in the nervous system both in flies and in humans. We performed systematic identification of the targets of Gαo signaling by combination of massive whole genome/proteome screenings, identifying numerous targets of this G protein, many of which are involved in vesicular trafficking. We further discover the dual localization of Gαo to the plasma membrane (PM) and the Golgi apparatus, conserved from insects to humans and required for formation and elongation of protrusions from a variety of cell types (e.g. neurites in neuronal cells). In humans, a set of dominant *de novo* mutations in Gαo underlies severe pediatric encephalopathies. Introduction of respective mutations in *Drosophila* Gαo recapitulates some of the disease phenotypes and suggests that, depending on the exact mutation, the PM vs. the Golgi function of the protein are differentially affected. Combining *Drosophila* genetics with cell studies, we work to test the hypothesis that mutations targeting mainly the canonical PM-mediated signaling of Gαo induce the subset of encephalopathies dominated by epileptic phenotypes, whereas mutants affecting predominantly its Golgi role underlie the subset of disease with neurodevelopmental delay, intellectual disability and movement disorders.

Poster 17

Developmentof a new automatic platform for continuous monitoring of the Cilia Beating Frequency (CBF)

A. Roux[1], *L. Gomez Baisac*[1], *N. Simonnet*[2], *X.-Y. Huang*[2], *S. Constant*[2], *L. Stoppini*[1]

[1]*Tissue Engineering Laboratory, HEPIA HES-SO, Geneva,* [2]*Epithelix SARL, Plan-Les-Ouates, Switzerland*

A number of methods have been developed for the measurement of CBF from ciliary epithelial cells. As alternative to animal testing, *in vitro* human airway epithelium made of primary cells is widely used for the prediction of inhalation toxicity of airbornes substances or efficacy inhalable treatments. For most of the applications, user have to remove wellplates from the incubator to perform the data acquisition by imaging. This step bring a bias in the result and is time-consuming. Then, the data treatment can be performed offline by a software afterward to measure the CBF which is a key marker of viability and health in epithelial lung cells.

Detection of long term effect is a challenge in *in vitro* culture, and users usually record data at specific time-points which is time and samples consuming. Herein we have developed an automated robotic platform combined with a low-cost camera able to perform continuous monitoring of CBF.

Our platform, alimented by battery or by power cable (USB), stays inside an incubator and can be controlled via any other computer. It can acquire single or multiples images but also single or multiples videos. Data are then stored locally and are accessible from any computer via internet.

This platform has been initially developed for 24 well plate format and could use cell culture insert (Transwell® type). The validation of the system has been performed by comparison with standard microscope using human airway epithelium reconstituted *in vitro*. Our platform measure CBF with good precision compared to the other system but more importantly allowing for the first time kinetics recording of

frequency giving insight of temporal mechanisms. Reference inhibitors or activators of CBF have been tested highlighting the applicability domain of this new platform for evaluation of inhaled compounds on cilia. In all cases, results were similar to standard method but now allowing time-course effect visualization . Such platform is a new useful tool for scientist working with the airways epithelium.

Poster 18

Bile salts regulate CYP7A1 expression and elicit a fibrotic response in 3D liver microtissues

C. Messner, L. Mauch, L. Suter-Dick

Institut für Chemie und Bioanalytik, Fachhochschule Nordwestschweiz Hochschule für Life Sciences, Muttenz, Switzerland

Introduction: Disrupted regulation and accumulation of bile salts in the liver can contribute towards progressive liver damage and fibrosis. The molecular mechanisms of this process are still unclear. Here, we investigated the role of bile salts (BS) in the progression of cholestatic injury and liver fibrosis. To this end, we used a 3D multicellular human liver *in vitro* model comprising the cell lines HepaRG, THP-1 and hTERT-HSCs, representative of hepatocytes, Kupffer cells and stellate cells, respectively. This *in vitro* model recapitulates cell-type specific fibrotic responses that include hepatocellular injury, inflammation and activation of HSCs, ultimately leading to increased deposition of ECM. The implementation of such a system to evaluate cholestasis would broaden its field of use as an alternative method and thus further increase its impact towards reduction of animal experimentation.

Methods: In order to better differentiate the contribution of individual cells during cholestasis, the effects of BS were evaluated either on each of the three cell types individually or in 3D scaffold-free multicellular microtissues (MTs) comprising all three investigated cell types. Cellular responses were assessed by gene expression analysis of hepatocyte specific markers (albumin, CYP7A1), cytokines (IL-6, TNF-α and TGF-β1) and the stellate cell activation marker αSMA. Moreover, release of the

liver specific miR-122 and deposition of extracellular matrix were also measured.

Discussion: Our data corroborate the toxic effects of BS on HepaRG cells and also indicate that non-parenchymal cells (THP-1 and hTERT-HSCs) cultured in monolayer respond to BS exposure with a slight inflammatory response. However, BS failed to directly trigger the activation of hTERT-HSC in monolayer cultures. Remarkably, using liver MTs composed of the three cell types, we could demonstrate that low concentrations of BS led to liver damage and triggered a fibrotic response characterized by hepatocellular injury, inflammation and stellate cell activation. This strongly suggests that the involvement of several cell types is necessary to achieve the BS-triggered activation of HSC. Moreover, BS were capable of down-regulating CYP7A1 expression.

Conclusion: BS regulate CYP7A1 expression in the human liver MTs and prolonged exposure results in cholestatic injury eliciting key events involved in fibrosis progression.

Poster 20

Lung alveoli-on-chip: the new generation of air-blood barrier in vitro model

P. Zamprogno[1], S. Wuethrich[1], S. Achenbach[1], J.D. Stucki[1], N. Hobi[1], N. Schneider-Daum[2], C.-M. Lehr[2], H. Huwer[3], R.A. Schmid[4], O.T. Guenat[1]

[1]ARTORG/ OOC-Technologies, University of Bern, Bern, Switzerland, [2]Drug Delivery (DDEL), Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, [3]Department of Cardiothoracic Surgery, Völklingen Heart Center, SHG Clinics, Völklingen, Germany, [4]Department of General Thoracic Surgery, University Hospital of Bern, Bern, Switzerland

Standard *in vitro* models poorly reproduce the microenvironment and the complex architecture of the lung parenchyma. The lung-on-chip, new advanced lung *in vitro* models, are emerging as predictive tissue modelling tools and as a credible alternative to animal testing. These

micro-engineered systems are able to reproduce the cellular composition and the rhythmic breathing motions of the air blood barrier. However, most of them fail to mimic the molecular composition and the intrinsic stiffness of the native air-blood barrier extracellular matrix. We report here about a new lung alveolar barrier model mimicking an array of alveoli with in-vivo like dimensions, based on a biological membrane. This new biological membrane is made of collagen and elastin, two of the main components of the lung extracellular matrix, which provide the elasticity to the tissue. It is supported by a hexagonal gold mesh mimicking the physiological dimension of the lung alveoli. The fabrication process is simple, reliable, and it is easy to use. The resulting membrane has a homogenous thickness of only few micrometers. It is also porous, flexible and optically transparent.

The membrane offers a good support for cells to grows and proliferate. Human primary alveolar epithelial cells from patients with primary human lung endothelial microvascular cells were successfully co-cultured for up than three weeks on the membrane. The cells were able to form a tight barrier as seen by the expression of typical tight junction marker ZO-1, even in the physiological air-liquid interface condition. Moreover, the human lung primary cells were successfully stretched at physiological range to mimic the breathing motions.

In conclusion, this new advanced model reproduces some keys features of the lung alveolar environment in terms of structure, extracellular matrix composition, barrier functions and dynamic microenvironment. It allows recreating an air blood barrier without any artificial layer between the epithelial and the endothelial cells. Moreover, the long-term stability of the membrane and its entirely biological nature makes this model a promising tool for drug discovery and, at long term, will help to reduce animal tests.

Poster 23

Alternative ex-vivo and in vitro models for studying human placenta function

L. Zurkinden[1,2], *S. Kallol*[1,2], *J. Zaugg*[1,2], *C. Albrecht*[1,2]

[1]*Institute of Biochemistry and Molecular Medicine, University of Bern,* [2]*NCCR TransCure, Swiss National Centre of competence in Research, Bern, Switzerland*

Serving as the interface between the maternal and fetal environment, the human placenta is centrally involved in the nutritional, excretory and respiratory exchange between the mother and the fetus, as well as in hormone synthesis and immune protection. In terms of maternal-fetal communication, active transport processes and passive diffusion across this trophoblast barrier are the principal transfer mechanisms for supplying selected nutrients into the fetal blood and removing waste products back to the maternal blood circulation.

To investigate the bidirectional transport across the trophoblast layer, our laboratory uses two alternative ex-vivo and *in vitro* models. The ex-vivo dual perfusion model of the human placenta offers an overview of substrate movements in an integrated functional cotyledon, but selected transport processes occurring at specific cell levels cannot be delineated.

To reflect physiological properties of the specific cells occurring at the barrier between maternal and fetal cells, we established a confluent primary trophoblast monolayer model using a pre-coated polycarbonate Transwell system. This model is appropriate for exploring the maternal-fetal exchange of endobiotics as well as xenobiotics across the trophoblast layer. Thereby it may not only provide new insights into bidirectional nutrient and drug transfer between the maternal and fetal compartment, but also can be applied to study intracellular metabolism, paracellular contributions and regulatory mechanism influencing the vectorial transport of molecules. Extension of this system to pathological trophoblasts isolated from diverse gestational diseases will help to better understand the underlying maternal or placental dysfunction and its impact on the fetus.

In this context our group successfully used these two systems to analyze the human placenta secretion of apolipoproteins and steroids. Thus, using the perfused placenta model we detected that apoE concentrations were significantly higher at the maternal compared to the fetal side. These results were confirmed on cellular level using the trophoblast monolayer system. Applying these models as alternatives to animal experimentation we were able to demonstrate that the human placenta plays an important role in maternal and fetal cholesterol homeostasis via secretion of anti-atherogenic apos.

Poster 26

EU-ToxRisk knowledge infrastructure - effective sharing of data, results and knowledge on new approach methods

L. Farcal[1], T. Exner[1], A. Hersey[2], M. Brajnik[1], U. Sarkans[2], M. Pastor[3], B. Hardy[1]

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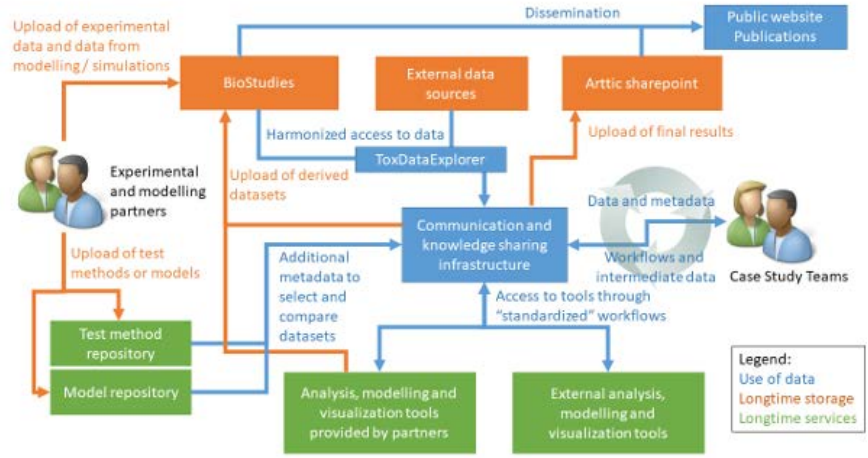
EU-ToxRisk - An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century - is a collaborative project funded by Horizon 2020, the EU Framework Programme for Research and Innovation. Its complex structure with almost 40 partners requires effective solution for sharing of data, knowledge and tools, first between consortium partners and later with the complete scientific community.

The Knowledge Infrastructure (KI) of EU-ToxRisk is designed as an one-stop shop effectively organising data and knowledge sharing and facilitating the usage of *in silico* prediction and risk assessment workflows. It builds the links between, on the one hand, the data and tool providers and, on the other hand, the read-across experts and risk assessors as the consumers of the data and users of the provided tools. The KI thus supports the ambitious goal to generate and have adopted new testing strategies and read-across applications for the assessment

of human health risks that are based solely on *in vitro* and *in silico* new approach methods (NAMs).

To be able to serve the requirements of all stakeholders, the KI consists of different modules that include long-term data storage solutions, linked visualisation and modelling tools, *in vitro* and *in silico* methods repositories, case studies and Adverse Outcome Pathway collaborative sections. One of its central components is the ToxDataExplorer (based on EdelweissDataTM technology), which makes available the data centrally stored on BioStudies via advanced application programming interfaces (APIs). This technology allows customised searching and filtering even across different datasets and enables a direct access and use of the data in a wide range of analysis or modelling tools, workflows and programming languages.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002 (EU-ToxRisk).



EU-ToxRisk Knowledge Sharing Platform overview

Poster 27

Validation of cell line model to study intervertebral disc neo-innervation associated with discogenic pain

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AO Research Institute, Davos, Switzerland

Introduction: Cell line is a widely used animal-free model, but its relevance to the behaviour of primary cells or cells within native tissues requires validation. [1] Deep neo-innervation has been observed in degenerative intervertebral discs where oxygen level is low (hypoxia). To study the effect of hypoxia on neurite outgrowth, the dorsal root ganglion (DRG) derived cell line ND7/23 was compared with primary DRG cell culture and DRG explants.

Methods: ND7/23 cell line was cultured under 2% (hypoxia) and 20% oxygen. Length and proportion of neurite longer than 30 µm were analysed at 2 and 6 days. Primary DRG cells and explants were isolated from freshly euthanized rabbits from other studies according to the "Reduce" principle of the 3R and cultured under 2% and 20% oxygen. Neurite outgrowth of primary cultures was analysed using the ´Sholl´ method [2] at 2 days and outgrowth of rabbit DRG explants was measured at 4 days regarding both length and frequency. Cell viability was evaluated in both cell line and primary culture using live and dead staining. Images were analysed using ImageJ. $p < 0.05$ was considered statistically significant.

Results and discussion: Neurite outgrowth length was increased by hypoxia in all cultures (cell line, primary cell and explant) (Figure 1). On the other hand, outgrowth frequency was reduced by hypoxia in explant culture, increased in primary culture and not significantly changed in ND7/23 cells (proportion of cells with outgrowth was not significantly different between oxygen levels). (Figure 2). Neuronal necrosis was increased by hypoxia in DRG primary cells but not cell line (Figure 3) which may be responsible for the hypoxia-reduced outgrowth frequency in DRG explant cultures.

Conclusions: The response of cell line to hypoxia showed the same trend comparing with primary DRG cell and explant cultures in terms of outgrowth length. However, cell line failed to predict the influence of hypoxia on outgrowth frequency in the primary DRG cell and explant culture. This highlights the relevance of more advanced explant culture in studying the interactions between the intervertebral disc and the surrounding neural structures and the "Reduction" principle of the 3R toward this purpose.

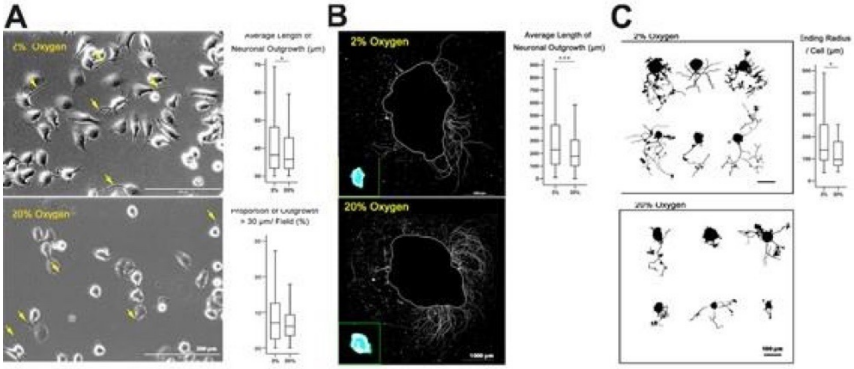

Fig.1: Influence of hypoxia on neurite outgrowth. (A) 'NeuronJ' (Fiji) analysis of ND7/23 cell line regarding neurite outgrowth length under 2% and 20% oxygen. Mann-Whitney test between 2% and 20% oxygen, *: p<0.05, n=362 and 364 cells from 3 independent experiments (each with 6 replicates) for 2% and 20% oxygen. (B) 'Simple neurite tracer' (Fiji) analysis of neurite outgrowth length from rabbit DRG explant after culturing in 2% and 20% oxygen for 4 days (Significant outgrowth developed at 4 days for most explants). * * *: p<0.001 by Mann-Whitney test, n=230 and 1131 nerve fibers from DRG explants from 4 rabbit donors (each donor 3-4 explants) for 2% and 20% oxygen respectively. (C) 'Sholl' (Fiji) evaluation of single rabbit DRG neuronal outgrowth length represented by ending radius after culturing in 2% and 20% oxygen for 4 days (Outgrowths intersect with each other if culture time is longer than 2 days). *: p<0.05 by Mann-Whitney test, n=25 and 27 cells from 2 rabbit donors for 2% and 20% oxygen respectively.

Fig.1: Influence of hypoxia on neurite outgrowth.

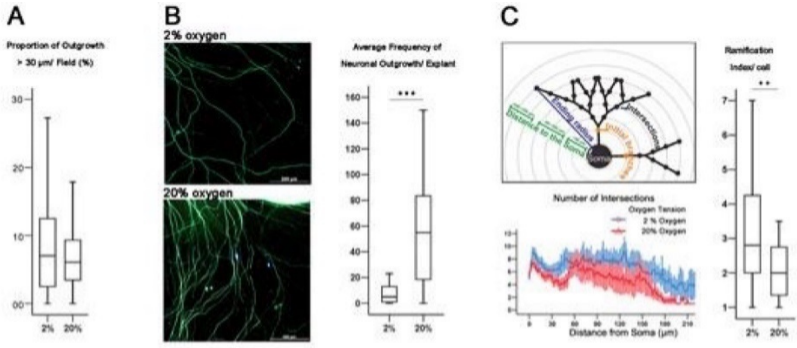

Fig. 2: Influence of hypoxia on the frequency of neurite outgrowth. (A) No significant difference was detected in proportion of ND7/23 cell line with outgrowth longer than 30 µm combining 2- and 6-day data. Mann-Whitney test p=0.375, n=141 and 138 fields from 3 independent experiments for 2% and 20% oxygen. (B) Neurite outgrowth frequency per DRG explant was significantly reduced by hypoxia treatment. Immunofluorescence staining using NF200 primary antibody. * * *: p<0.001 by Mann-Whitney test, n=22 and 20 explants for 2% and 20% oxygen respectively from 4 rabbit donors. (C) Branching of neurite outgrowth per neuron was analysed by the increased outgrowth intersection with consecutive sampling circles and can be quantified using 'Ramification Index'. Hypoxia culture of 2 days showed higher ramification index than 20% oxygen. * *: p<0.01 by Mann-Whitney test, n=25 and 27 cells from 2 rabbit donors for 2% and 20% oxygen respectively.

Fig. 2: Influence of hypoxia on the frequency of neurite outgrowth.

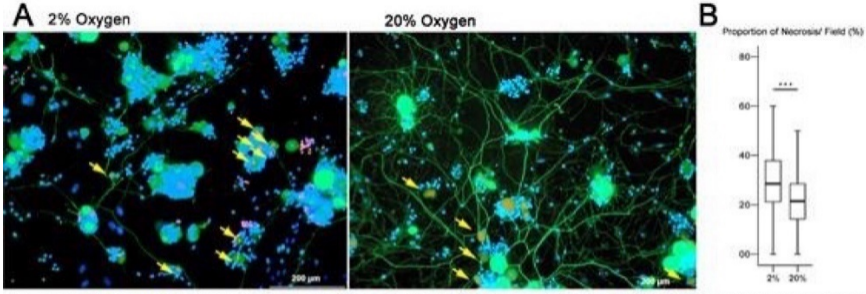

Fig. 3: Influence of hypoxia on neuronal necrosis at 4 days of culture. (A). Ethidium-homodimmer-1 staining, neurofilament (NF200) immunofluorescence and Hoechst staining was used to evaluate neuronal necrosis. (B). Hypoxia culture showed significantly higher necrosis per field. * * *: p<0.001 by Mann-Whitney test, n=210 and 223 fields of DRG cell imaging pooled by 2 rabbit donors for 2% and 20% of oxygen respectively.

Fig. 3: Influence of hypoxia on neuronal necrosis at 4 days of culture.

Poster 28

Development of a fibrosis on-chip companion diagnostic tool

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Introduction: Fibrotic diseases account for an increasing burden of disease-associated morbidity and mortality worldwide. The fibrosis hallmark is the stiffening and loss of function of diseased tissues due to the development of scars. It can affect many organs in the body (lung, liver, heart and many others) and remains difficult to treat, because the drug response is often patient specific. Fibrosis is not only an etiology but also a major propagating factor of the pathogenesis of many diseases. The intricate underlying biomechanism and plethora of involved cells as well as metabolic molecules are subject to many research projects but remain not fully elucidated. The increased deposition of extracellular matrix is mediated through activated stellate cells (liver) and myofibroblasts (lung, heart). These cells have been identified to play a key role in the development and progression of fibrosis. Therapeutic approaches to tackle this disease are also manifold, for example removing the cause if identifiable, and the administration of drugs, whereas the latter often aims at the modification of the patient's stellate cells/myofibroblasts. Both, diagnosis and therapy, are still subject to many uncertainties, and the success of pharmaceuticals differs from patient to patient.

Methods: In this research work we aim at developing a companion diagnostic tool while taking advantage of the on-chip microfluidic technologies. Applying a given pressure onto a thin, elastic and soft membrane results in a measurable deflection, which heights varies depending on the stiffness of the cell culture on top.

Results: Preliminary results using activated lung fibroblasts and stellate cells show that the production of collagen can be detected by this method, when comparing metabolically activated with not activated conditions. Through the use of a polydimethylsiloxane membrane as

culture substrate for five to six days with subsequent deflection measurements using an upright microscope in reflective mode, a facile approach with minimal equipment requirements could be established.

Conclusions: This should allow for a patient-specific determination of the degree of fibrosis on a cellular level, while at the same time enable optimized drug testing and therefore not only refine, but also reduce and ultimately even replace animal testing.

Poster 29

Towards in-silico behavioral neuroscience

L. Restivo

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Data collections are increasingly adopted for *in-silico* experiments in neuroscience. Behavioral neuroscience is recently taking advantage of innovations in data-driven approaches to further our insights into how the brain supports behavior and cognitive processes. On one hand, machine learning approaches have significantly improved non-invasive tracking of complex behaviors and the discovery of novel behavioral motifs. On the other hand, data-driven approaches promote the discovery of novel task-related latent behavioral variables that better predict neural population dynamics. However, replacement of animals in behavioral neuroscience poses a great challenge to the scientific community. Here we present a resource to further promote the adoption of *in-silico* experiments in behavioral neuroscience: the "Open-Water Maze" (OWM). The OWM is a digital reconstruction of a real environment (i.e. a water maze) used to assess spatial memory in mice. A set (N = 16) of pictures (260 degrees, 22.5 degrees increment) was acquired from each cell (N = 45) of a grid overlaid on the pool to obtain the estimated field of view of the rodent navigating the water maze. The OWM´s visual environment can be used for testing deep-reinforcement learning algorithms and lays the foundation for a close comparison between the performances of biological (i.e. mice) and artificial agents. In addition,

the entire OWM's environment (i.e. extra-maze cues) is embedded in a collection of vectors coding for the absence/presence of salient cues in each frame, providing an information-theoretic framework for studying exploration Vs exploitation strategies *in-silico*.

Poster 31

Fish cell lines of rainbow trout as alternatives to fish in environmental risk assessment

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Introduction: Millions of fish are used every year in the safety testing of chemicals and water samples. Three OECD certified tests are most commonly used, e.g. under the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation: fish acute toxicity test (OECD203), the fish early life stage test (OECD 210) and a test to determine chemical bioconcentration factors (OECD305). In order to start reducing or even replacing these tests, we develop experimental strategies that evolve around fish cell lines.

Methods: Depending on the physico-chemical properties of chemicals and the physiology of fish, chemicals are assumed to be taken up mainly via the gill or the intestine. The liver, on the other hand, is considered as a major site for chemical biotransformation. Thus, we focus our research on three cell lines derived from, respectively, rainbow trout (*Oncorhynchus mykiss*) gill, intestine and liver: the RTgill-W1, RTgutGC and RTL-W1. Exposure of cells to chemicals is combined with quantification of chemical exposure and with mechanistic computational models as appropriate.

Results: We have been able to design an assay, based on the RTgill-W1 cell line, to predict fish acute toxicity. This assay has been independently validated and has undergone round-robin testing, leading to adoption as

standard by ISO (ISO21115) in April 2019; evaluation by the OECD is underway. Moreover, a combination of RTgill-W1 cell proliferation and physiology-based and growth modelling led to a procedure that provides estimates of reduced fish growth upon chemical exposure. Finally, the gill, intestinal and liver cell line combined are currently under investigation to provide chemical clearance rates for *in vitro-in vivo* extrapolation for bioaccumulation prediction.

Conclusions: If employed in an integrative strategy, cell lines of fish can be an invaluable component of test designs that aim to reduce or replace fish in environmental risk assessment.

Poster 32

Automated high-content phenotyping of the nematode Caenorhabditis elegans: application to the toxicity assessment of perovskites materials

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[1]Laboratory of Microsystems, EPF Lausanne, [2]Nagi Bioscience SA, [3]Center for Integrative Genomics, Université de Lausanne, [4]Laboratory of Physics of Complex Matter, [5]Laboratory of Integrative Systems Physiology, EPF Lausanne, Lausanne, Switzerland

The booming need for drug and chemical testing cannot be satisfied today because: (i) the use of *in vivo* models for biological testing is more and more under scrutiny, due to increasing costs of these tests and rapidly growing pressure by the public opinion and governments for reducing and replacing animal tests with more ethical solutions; (ii) current *in vitro* alternatives to animal testing are based on simplified cellular models, which cannot capture the complex interplay among cells in living organisms and are thus unable to provide responses at organismal level. Here we describe an innovative platform which combines the use the nematode *Caenorhabditis elegans* as validated *in vivo* models for drug/chemical screening, with the first laboratory device for their fully automated *in vitro* culture, treatment and multi-parametric

analysis[1-3]. Our microfluidic device allows automated high-content phenotyping of *C. elegans*, via accurate control of worm culture conditions and real-time automated monitoring of multiple physiological parameters (Fig. 1). This screening format could be readily used to identify toxicity mechanisms of substances, through specific phenotypic responses in the worms, within only 4 days[4]. We employed our platform for screening organo-metallic photovoltaic perovskites, which are making a breakthrough in light to electricity conversion efficiency. Because of their heavy element content, there is a concern for health hazards related to their production, transportation and their leakage into the environment in case of failure of the installed photovoltaic devices. Our results show that two of the most studied photovoltaic perovskites, CN3NH3PbI3 and CN3NH3SnI3, may significantly impact development, fertility and survival of *C. elegans* when suspended/dissolved in liquid medium, even at relatively low concentrations (Fig. 2). Given the increased frequency at which application-oriented novel compounds emerge every year, e.g. in the domain of new nanomaterials, our technology is expected to become a new important tool for the rapid *in vivo* assessment of potential health hazards of new compounds before any large-scale production. In conclusion, we propose an innovative solution for rapid identification of toxic compounds and their potential mechanism of toxicity, using a biological model that perfectly bridges the gap between *in vitro* and *in vivo* assays.

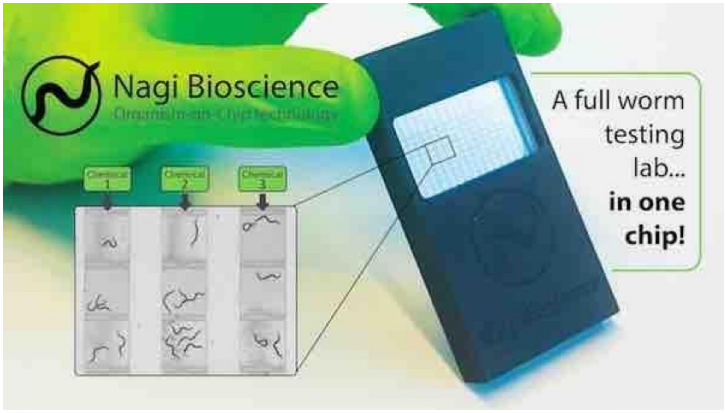

Figure 1. Picture of the microfluidic cartridge and the confinement of the C.elegans

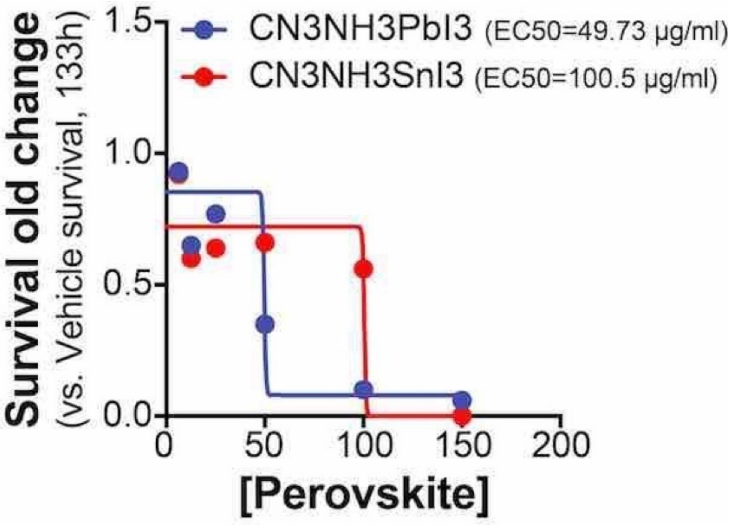

Figure 2. Dose response curves for the worm's survival.

Poster 37

Fishy alternatives: recent developments to reduce the need for in vivo fish tests in the regulatory risk assessment of chemicals

H. Segner

Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

National and international regulations require the use of *in vivo* fish tests to assess the risk of chemicals and effluents for the environment. In fact, fish are the most frequently used vertebrates in environmental risk assessment. A key parameter that has to be determined in this context is the bioaccumulation of chemicals in fish. The corresponding *in vivo* fish test, which is laid down in the OECD Test Guideline (TG) 305, is under discussion because of its high animal usage. For instance, under the new European chemical regulation REACH it is expected that more than 500 000 fishes will be used in the coming years for performing the OECD TG 305. Thus, there is an urgent need for alternative methods. . A key process influencing bioaccumulation is the enzymatic biotransformation of chemicals in the organism. This process takes place primarily in the liver. It has been suggested that *in vitro* assays using liver preparations (isolated liver cells or subcellular fractions) should be able to determine the metabolic turnover of chemicals in the liver and to predict the *in vivo* biotransformation rates and bioaccumulation in the intact animal. In this way, the *in vitro* ways could lead to a reduction of the *in vivo* bioaccumulation testing. Over the least 10 years, an international cooperative research effort has been undertaken in order (i) to establish *in vitro* methodologies using isolated liver cells or subcellular preparations form fish liver, (ii) to explore their capability to measure chemical biotransformation *in vitro* and to predict the *in vivo* bioaccumulation in fish, and (iii) and to translate the results of this research into a standardized test guideline that can be implemented for regulatory applications. In spring 2018, this was successfully achieved with the publication of two OECD Test Guidelines (319 a, b) on *in vitro* fish liver assays. This is the first time that *in vitro* assays have

been introduced as alternatives to *in vivo* fish tests in the regulatory risk assessment of chemicals.

Poster 44

Development of phage display based minirecombinant bodies to investigate the function of beta1-integrin acetylation in CAFs

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Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, Geneva, Switzerland

Introduction: Undoubtedly, monoclonal antibodies (mAbs) have been a safe bet for decades in research and therapeutics fields. However, mAbs but also polyclonal antibodies feature several drawbacks such as the immortalization of B-cells for hybridomas and ascites production in animal models. Furthermore, when it comes to the broad spectrum of post-translational modifications (PTMs), the efficiency of mAbs was extensively demonstrated for phosphorylated proteins, but only few examples of mAbs specifically detecting other PTMs have been described. As recent evidences emphasize the relevance of the metabolic reprogramming in cancer, acetylation has emerged as a reversible PTM directly mirroring the metabolic environmental level, thereof not only involved in nuclear histones regulation. We, thus, hypothesized that cancer associated fibroblasts (CAFs) mediate the ectopic stiffness of the ExtraCellular Matrix (ECM) through the acetylation of the β1-integrin. In order to tackle the many hurdles of mAbs production, we resorted to the phage-display technology to develop exclusively specific acetylated- β1-integrin recombinant single chain antibodies.

Methods: Accordingly, we developed miniRecombinant bodies (mRbs, or single chain antibodies) that were screened for their specific interaction with an acetylated β1-integrin peptide. In addition, selected clones were further expressed as intracellular site-specific reagents to validate the physiological function of β1-integrin acetylation.

Results: When expressed in the cytoplasm, the mRbs blocked fibronectin fiber assembly, labeled extracellular cross-linked and cytoplasmically stripped fibrillary adhesions. Furthermore, deacetylase inhibitors and fluctuations in serum and glucose levels modulated fibers synthesis in a b1-integrin acetylation-dependent manner.

Conclusion: Our mRbs provide the first evidence of an effective detection of the acetylated β1-integrin and its functional role. This unprecedented study may set the stage for therapies targeting the host stroma in human cancer but also in various fibrosis related diseases, as well as showing the potential of recombinant antibody screening technology for mass production that could replace animal-based immunization techniques.

Debate session

12h00 – 12h45

Nonanimal-derived affinity-reagent antibodies

Andreas Plückthun (UZH) and Pierre Cosson (UniGe)

Antibodies for research in biology and biomedicine are still mostly made from immunized animals, typically mice and rabbits. On the other hand, technology has existed for over two decades to produce human antibodies recombinantly by biotechnological methods. All antibodies for human therapy — the ones with the highest demands for quality — are made with this technology. The main reasons why research antibodies are lagging so far behind is a lack of understanding among researchers about today's possibilities, and the business models of traditional manufacturers of reagent antibodies. Besides reducing the need for animal experimentation, recombinant antibodies are essential to generate reproducible results. Only with a recombinant antibody can two distinct experimentators be certain that they are using the exact same reagent and compare meaningfully their results.

For researchers still using traditional antibodies, it is now time to switch to recombinant antibodies. We will describe the available tools and services allowing to generate recombinant antibodies and next generation binding reagents against specific targets, to sequence existing monoclonal antibodies in order to convert them into recombinant reagents, to identify existing recombinant antibodies and order them.

The discussion will highlight the problems with today's reagent antibodies and possible solutions, including next-generation reagents that even surpass what is possible with traditional antibodies.

Further reading:

Bradbury, A., and Plückthun, A. (2015). **Standardize antibodies used in research**. *Nature* **518**, 27-29.

Bradbury, A. R., and Plückthun, A. (2015). **Getting to reproducible antibodies: the rationale for sequenced recombinant characterized reagents**. *Protein Eng. Des. Sel.* **28**, 303-305.

https://www.unige.ch/medecine/antibodies/

https://www.bioc.uzh.ch/de/forschung/core-facilities/high-throughput-binder-selection-platform/

Session II. Reduction

12.45 - 13.45	*Lunch break & REDUCTION poster presentations*	
13.45 – 15.10	**Session II: Reduction**	Co-chairs: Hanno Würbel & Armand Mensen
13.45 – 14.05	Standardization and the reproducibility paradox in animal research	Hanno Würbel University Bern
14.05 – 14.25	Multi-spectral optoacoustic tomography at the service of the 3Rs	Daniel Razansky UZH & ETH, Zürich
14.25 – 14.45	Avoiding statistical power shortage in a 3R perspective	Romain-Daniel Gosselin CHUV, Lausanne
14.45 - 15.05	AniMatch, an innovative web-based platform to share organs and tissues.	Annemarie Lang, Charité, Berlin, Germany Young Scientist

Oral presentations - Reduction session

13h45 – 14h05

Standardization and the reproducibility paradox in animal research

Hanno Würbel, University of Bern, Switzerland

Reproducibility in animal research is alarmingly low. Various potential causes of poor reproducibility have been identified, including poor scientific rigor, low statistical power, analytical flexibility, and publication bias. However, the reproducibility of a result is also a function of its external validity. Unless results are robust against common sources of variation between independent replicate studies, they will not be reproducible. In animal research, effects of experimental treatments usually vary depending on the phenotype of the animals. Because the phenotype depends also on the environment of the animals, small differences in the environment between replicate studies can produce conflicting results. Therefore, systematic variation (heterogenization) rather than more rigorous standardization is required to improve reproducibility. Based on theory, simulations with existing preclinical animal data, and experimental results, I will show how heterogenization of study populations improves the external validity and, therefore, reproducibility of results, without a need for lager sample sizes. Using more heterogeneous study samples will be crucial to avoid wasting animals for inconclusive research.

14h05 – 14h25

Multi-spectral optoacoustic tomography at the service of the 3Rs

Daniel Razansky, UZH & ETH, Zürich, Switzerland

Rapid progress in the development of Multi-Spectral Optoacoustic Tomography (MSOT) technology has enabled unprecedented insights into in vivo biological dynamics and molecular processes. This novel functional and molecular imaging modality is capable of entirely non-invasive longitudinal observations on the same animal at penetration and spatiotemporal scales not covered by modern optical microscopy methods. Biomedical applications are explored in the areas of functional neuro-imaging, fast tracking of agent kinetics and biodistribution, cardiovascular research, monitoring of therapies and drug efficacy as well as targeted molecular imaging studies. MSOT further allows for a handheld operation thus offering new level of precision for clinical diagnostics of patients in a number of indications, such as breast and skin lesions, lymph node metastases, thyroid conditions and inflammatory bowel disease.

14h25 – 14h45

Avoiding statistical power shortage in a 3R perspective

Romain-Daniel Gosselin, CHUV, Lausanne, Switzerland

In the context of the 3Rs, the principle of Reduction may seem in apparent contradiction with the need of sufficiently powered statistical design. However, this legitimate concern masks the fact that statistical power is based on other actionable parameters than sample size. This lecture aims at offering a brief overview of various possibilities in the experimenter's arsenal that can be used to reconcile the principle of Reduction with high standards in statistical design.

14h45 – 15h05

AniMatch, an innovative web-based platform to share organs and tissues

A. Lang[1,2]

[1]*AniMatch UG (Haftungsbeschränkt),* [2]*Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany*

During the last years, the number of animals that have been used for scientific purposes in Europe has increased. The itemization of categories revealed scarcely used potential to reduce animals that are not used in experiments but for the collection of tissue and organs. The development and deployment of a web-based platform that enables scientists to connect and share organs and tissue of killed animals would directly address the request of the EU directive as well as exploit the existing potential to reduce animals and save the biological resources that are gained. Therefore, we have developed AniMatch, an innovative web-based platform that allows scientists to register and publish or search for offers to facilitate the multiple use of killed animals. To publish an offer the providing party must quote the species, type and if necessary, the genetic background as well as the number, age, sex, the organ or tissue that is used for own purposes and the timeframe for the killing. The seeking party can search a list including filters for the species and a geographical radius and request while quoting the number of animals and organs or tissue in need. With completion of the request the contact information is exchanged between both parties who are now able to arrange the details of the transfer. Since 2017, we have been providing an adapted version of AniMatch for the Switzerland (swiss.animatch.eu) which was built in cooperation with the University of Zurich. Optimizations of our service have been performed after intensive discussions with animal welfare officers. Subsequently, we have implemented two safety barriers in the registration process in order to avoid abuse. The measures include approval of affiliation and account activation by the designated animal welfare officer. Furthermore, we integrated a complex matching system that focuses on the verification of the different microbiological units (hygienic management system) that must be considered during the sharing

process. Besides the moral exculpation of scientists, AniMatch provides a cost-efficient way to use existing infrastructure and to conserve resources in accordance with reducing lab animal usage.

Poster presentations – Reduction session

Poster 10

In new dimensions! - 3D OCT via post-imaging digital data processing

N. Denk[1], *P. Hasler*[2], *P. Maloca*[3], *T. Schnitzer*[1], *T. Singer*[1]

[1]*Pharmaceutical Sciences, Hoffmann La Roche Ltd.,* [2]*University of Basel,* [3]*IOB, Basel, Switzerland*

Introduction: Optical coherence tomography (OCT) is a routinely used imaging tool used in ocular preclinical safety assessment. Image acquisition itself is non-invasive, however animals are anesthetized for the procedure. There are several limitations to this imaging technique: assessment of surfaces or spatial relationships is not possible. Other structures cannot at all be assessed given signal loss in deeper structures, such as e.g. the choroid of certain species. This limits the use of OCT depending on the type of pathology, thus increasing the need for interim sacrifices throughout the study time course. Goal of this project was to overcome these shortcoming: by advanced digital data processing in order to maximize the readout possibilities for *in vivo* imaging versus post-mortem analysis.

Methods: A semi-automated algorithm was developed to remove speckle noise while preserving the structure and to extract, display and measure the volume of various intraocular structures including the choroid. Subsequent 3D rendering was applied to various ocular structures followed by a qualitative and quantitative analysis.

Results: New advancements in image analysis now allow for a great enhancement of assessable detail *in vivo*. Methods were validated by great reproducibility in repeated assessments by blinded reviewers.

Conclusion: OCT is considered current gold standard in ocular imaging, however thus far was limited to a qualitative readout and did not allow assessment of volumetric readouts and certain structures. We overcame these limitations by advanced digital data processing after

routine image acquisition. This now enables assessment of additional intraocular aspects and structures that were so far considered a "hidden space" *in vivo*. Ultimately, this refinement results in a direct reduction of animal numbers needed for a study, as interim sacrifices to assess a certain pathology throughout a safety study can now partially be replaced by enhanced *in vivo* readout capabilities.

Poster 11

The extracellular interactome of prostate cancer identifies secreted factors responsible for the tumor cell-immune cell crosstalk driven by different genetic backgrounds

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Prostate cancer is the second most commonly occurring cancer in men and the fourth most commonly occurring cancer overall. In 2018 the new cases are 1.3 million. There is an urgent need for development of new effective drug strategies that can counteract the onset of resistance to androgen deprivation therapy and metastasis. Tumor microenvironment plays a fundamental role in the progression of cancer as demonstrated by the emergence of cancer immunotherapy. The genetic background can shape the immune response leading to an immunosuppressive microenvironment and cancer immunotherapy can be tailored for specific genetic alterations. Our aim is to identify novel secreted or transmembrane proteins expressed on the surface of the tumor cells from different genetic backgrounds that mediate the recruitment or skewing of myeloid cells, eliciting immunosuppression and tumor escape from the immune surveillance. In order to characterize the immune landscape of preclinical models of prostate cancer driven by different genetic alterations, we performed immunophenotyping coupled to polysome profiling of normal prostate and prostate tumor of $Pten^{pc-/-}$, $Pten^{pc-/-}$;TMPRSS2-ERG, $Pten^{pc-/-}$; CDCP1+, $Pten^{pc-/-}$; $TIMP1^{-/-}$ and$Pten^{pc-/-}$; $Trp53^{pc-/-}$. Pten and Trp53 loss and

TMPRSS2-ERG fusion are among the most common genetic alterations in prostate cancer. We matched the gene expression profiling from prostate cancer to the signature obtained from bone marrow-derived myeloid derived suppressor cells (MDSCs), in order to identify proteins upregulated in prostate cancer that can modulate MDSCs through the binding to their specific receptors. We are currently investigating the relevance of a set of receptors that we found upregulated in MDSCs by setting up a panel of *in vitro* experiments aimed to asses cell migration, T cell suppression activity and cytokine productions. The receptors that are able to increase migratory and suppressive capacity *in vitro* will be tested in preclinicals mouse models of prostate cancer. The identification of novel receptors involved in the recruitment or skewing of immunosuppressive myeloid cells into the tumor will potentially allow to design new immunotherapy strategies that, combined with exisisting chemotheraphy, might ameliorate the efficacy of prostate cancer treatment. In addition, the identification of specific, genetically-driven alterations, might allow to design personalized therapies.

Poster 12

Reduction in animal use for PK screening - a cassette dosing study with monoclonal antibodies or oligonucleotides in cynomolgus monkey enabled by innovative LC-MS bioanalytical methods

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Introduction: There is a growing need for pharmacokinetic (PK) screening of biotherapeutics and oligonucleotides to support the selection of drug candidates. In order to reduce the number of animals used for PK assessment, it is of great interest to administer multiple compounds at the same time to the same animal (so called cassette dosing), which can reduce the number of animals markedly. The use of cassette dosing is well established for small molecules, but it has been

more difficult to implement for biotherapeutics and oligonucleotides. The main reason has been the lack of specific bioanalytical assays to quantify multiple analytes in the same sample.

Methods: Here we present the case example of a cassette dosing with three monoclonal antibodies or two oligonucleotides to cynomolgus monkeys enabled by innovative LC-MS methods. Cynomolgus monkeys (n=3) were dosed intravenously with a cassette of three structurally related monoclonal antibodies at a dose level of 0.85 mg/kg each. Two oligonucleotides were administered subcutaneously as a cocktail at doses of 1, 3, and 9 mg/kg. In both cases, serum samples were collected and analyzed using LC-MS assays, which were developed and qualified to allow specific quantification of each co-administered analyte. The bioanalytical method for mAbs method involved proteolytic digestion and quantification of selective signature peptides identified in the hypervariable regions of the three antibody variants. For increased sensitivity, immunoaffinity extraction was performed, followed by UPLC chromatography and tandem MS detection. The bioanalytical method for oligonucleotides involved solid phase extraction to isolate the analytes from the biological matrix, followed by ion-pairing UPLC chromatography coupled with tandem MS detection.

Results: In both cases, PK samples could be successfully analyzed for the co-administered compounds and all pre-defined bioanalytical acceptance criteria were met. The pharmacokinetics of all test compounds could be well characterized.

Conclusions: LC-MS analysis enabled cassette dosing of monoclonal antibodies or oligonucleotides in cynomolgus monkeys with concomitant PK characterization of several molecules in the same animals, demonstrating that an animal reduction of 50-70% is feasible. The technique also enables the co-administration of well-characterized control drugs, to allow for normalization of PK results and characterization of inter-individual PK variability.

Poster 22

Reproducibility in the light of biological variation: implications for experimental design

B. Voelkl

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According to the prevailing view, the reproducibility crisis in pre-clinical animal research is caused by a lack of scientific rigor, low statistical power, and publication bias [1-3]. However, here I argue that ignorance of biological variation that we will encounter whenever conducting an experiment with living animals might be a major reason for irreproducibility of research findings. This biological variation is distinct from random noise or measurement error. Biological variation is the sum of genetic variation, environmentally induced variation, and gene-by-environment interactions. The response of an animal to a drug treatment depends, therefore, not only on the treatment but also on the current state of the animal, which is as much the product of past and present environmental influences as of its genetic background [4]. This represents a unique challenge to reproducibility in laboratory animal research. We can account for this by adopting a reaction norm perspective on treatment responses in animal experiments [5, 6]. This means to abandon the idea that we can estimate "true" population parameters and it entails a fundamental re-thinking of parameter estimation, statistical inference and interpretation of study results in laboratory animal research. Ignoring the implications of biological variation is likely to lead to spurious results that are idiosyncratic to the specific standardized laboratory conditions, thereby causing poor reproducibility. Irreproducible results generate the need for follow-up studies requiring further animals, or might—in the worst case—lead researchers into scientific dead ends, wasting even more animals for studies that cannot provide any benefits in terms of knowledge gains [7]. Implementing experimental designs that embrace biological variation can increase the external validity and the reproducibility of animal experiments and can, therefore, reduce the number of laboratory animals sacrificed and wasted for inconclusive experiments.

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5. Voelkl & Würbel (2016): Trends Pharmacol Sci, 37, 509-510.
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Poster 33

The effects of the rearing environment on measures of stress: a multi-laboratory study

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Introduction: Lack of reproducibility is a prominent problem in biomedical research, with the neuroscience field being no exception. Here, we hypothesize that inconsistencies between different studies depend partly on common differences between the rearing conditions of laboratory mice. Together with rigorous within-laboratory standardization, this may cause high between-study heterogeneity. In order to test this hypothesis, we will evaluate how differences in the environmental conditions (housing and husbandry) between different rearing laboratories affect the expression of stress responses in mice, from behaviour to the molecular level.

Methods: We will rear genetically identical mice (C57BL/6 mice from a single breeding stock) in 5 different laboratories before testing them in our laboratory for phenotypic differences induced by the different rearing environments. We will focus specifically on environmentally induced variation in HPA stress reactivity and anxiety related behaviour. To assess the biological basis of the expected phenotypic differences, a whole genome analysis of epigenetic changes in DNA methylation will be conducted.

Results: Our findings will reveal the range of phenotypic variation in stress response induced by common differences between the rearing conditions of laboratory mice and associated epigenetic changes could reveal candidate genes that may uncover molecular mechanisms of this variability.

Conclusion: The results will contribute to a better understanding of between-study variability in laboratory animal research and the mechanisms underlying such variability. These findings will also inform study design of laboratory animal research in view of better reproducibility of study outcomes.

Plenary lecture

15h10 – 15h40

Best practice and alternatives in education and training

Nick Jukes InterNICHE, Leicester, United Kingdom

The design of the curriculum for veterinary, medical and biology education and training involves choices about the tools employed to meet teaching objectives. Ensuring that the tools are the most appropriate requires clarity on teaching objectives and an awareness of developments in technology, educational practice and ethics. Harmful animal use, specifically animal experimentation, the dissection of purpose-killed animals, and other instrumental animal use, continues to be employed in some practical classes.

However, innovative and humane alternative methods are now widely available and increasingly are being implemented worldwide to achieve replacement and to enhance the acquisition of knowledge, skills and attitudes. This transition reflects a growing commitment to best practice and sustainability, an appreciation of the advantages of alternatives, and the demands of students, trainees and campaigners. Alternatives include non-animal tools such as advanced synthetic cadavers and training mannekins, 3D printed materials, software and virtual reality. They also include alternative approaches such as student self-experimentation, client donation programs for ethically sourced animal cadavers, case-based clinical learning opportunities with patients, and ethical field work. Examples of the range of alternatives employed within education and training for anatomy, physiology, pharmacology, clinical skills and surgery will be detailed.

Some of the myths and misunderstandings concerning animal use and alternatives in education and training will be uncovered and explained. The limitations and the re-definition of the 3Rs that is necessary for

these fields will be argued, and the differences between the training of students and that of scientists and technicians will be described. Teaching objectives and the lessons of the hidden curriculum will be explored, and published studies will provide further evidence of the multiple advantages of alternatives for students, teachers, the professions - as well as animals. Case studies will show that such tools and approaches are often no longer considered 'alternative', but the norm.

Session III. Refinement

15.40 – 16.10	*Coffee break & REFINEMENTposter presentations*	
16.10 – 17.45	**Session III: Refinement**	Co-chairs: Paulin Jirkof & Isabelle Desbaillets
16.10 – 16.35	How a culture of care can improve animal welfare	Paulin Jirkof University Zürich
16.35 – 16.55	Development of a sustained-release depot formulation of buprenorphine for pain relief in experimental animals	Viktoria Schreiner University Basel Young Scientist
16.55 – 17.15	Report of the FSVO 3R Symposium – Alternatives to CO_2	Thom Gent University Zürich
17.15 - 17.30	Investigating severity and analgesia in the acute Cerulein-induced pancreatitis mouse model.	Mattea Durst University Zürich Young Scientist
17.30- 17.45	One Europe: The challenge of consistency in severity classification	Anne Zintzsch University Giessen, Germany Young Scientist

Oral presentations - Refinement session

16h10 – 16h35

How a Culture of Care can improve animal welfare

Paulin Jirkof, Dept. Animal Welfare and 3Rs, University of Zurich

The term Culture of Care is used to indicate an organizational commitment to improve animal welfare, scientific quality, care of the staff as well as transparency for stakeholders.

Simply having animal facilities and resources which meet the legal requirements is not enough to ensure that appropriate animal welfare, care and use practices will follow. All those involved in the care and use of animals should be committed to the 3Rs principles and demonstrate a caring and respectful attitude towards the animals bred or used for scientific purposes. This includes an attitude based on an individual's positive and proactive mind-set and approach to animal welfare and humane science.

The talk will present key factors of a Culture of Care and discuss how they can facilitate refinement initiatives and improve animal welfare.

16h35 – 16h55

Development of a sustained-release depot formulation of buprenorphine for pain relief in experimental animals

V. Schreiner[1], P. Detampel[1], M. Durst[2], P. Jirkof[2,3], M. Puchkov[1], J. Huwyler[1]

[1]Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel, Basel, [2]Division of Surgical Research, University Hospital Zurich, University Zurich, [3]Department of Animal Welfare, University Zurich, Zurich, Switzerland

Introduction: Buprenorphine is a fast-acting semisynthetic opioid derivative, frequently used in veterinary medicine for post-surgical pain relief in mice and rats. Due to its short half-life, repeated injections are required to maintain analgesic effect. Consequently, animals are exposed to increased levels of stress and might suffer additional pain. As no depot formulation is available on the European market, the goal of this project is to develop a sustained-release formulation of buprenorphine to prolong the analgesic effect after surgical intervention in rodents.

Methods: The developed sustained-release formulation is based on drug-loaded microparticles. Various formulations were studied regarding their influence on *in vitro* drug release, prior to *in vivo* testing. The most promising formulation was administered subcutaneously to 32 female C57BL/6J mice to investigate the pharmacokinetic profile of buprenorphine over a period of 72 hours. Analgesic action was further assessed up to 48 hours after a single injection using a thermal sensitivity test (hotplate assay) and compared to the standard formulation Temgesic®.

Results: Serum concentrations indicate that the here developed depot formulation provides an adequate analgesic effect for at least 24 hours. After 48 hours, serum buprenorphine concentrations drop below the threshold where an antinociceptive effect can be expected. The hotplate assay confirmed a significant increase in withdrawal latency for the sustained-release formulation after 2, 12 and 24 hours when compared to the baseline. Furthermore, a significant increase in

withdrawal latency for Temgesic® could only be shown for the first time point of 2 hours. Afterwards, no significant difference to baseline was detected, confirming the short duration of action of less than 12 hours.

Conclusion: A promising sustained-release depot formulation of buprenorphine based on biodegradable microparticles was developed. Serum concentrations and thermal sensitivity assays show an analgesic efficacy of at least 24 hours, significantly prolonging the effect compared to the marketed standard formulation.

16h55 – 17h15

Report of the FSVO 3R Symposium – Alternatives to CO_2

Thom Gent, Vetsuisse, University of Zurich

The Swiss Federal Food Safety and Veterinary Office (FSVO) commissioned a research strategy aimed at identifying research priorities which will ultimately lead to the identification and implementation of stunning and killing methods which are more humane than the current practice of carbon dioxide. The research strategy targets mice, rats, pigs and poultry. In particular, the need for the consistent use of terminology across studies, a consensus and validation of behavioural testing for aversion and the distress caused by controlled atmospheric stunning were identified. The idiosyncrasies of each species in relation to these themes are discussed.

17h15 – 17h30

Investigating severity and analgesia in the acute Cerulein-induced pancreatitis mouse model

M.S. Durst[1], *M. Arras*[1], *P. Jirkof*[1,2]

[1]*Division of Surgical Research, University Hospital Zurich, University Zurich,* [2]*Department Animal Welfare, University Zurich, Zurich, Switzerland*

Introduction: Translational research heavily relies on mouse models to investigate acute pancreatitis. Pancreatitis is painful in humans and companion animals but the level of pain in the acute mouse Cerulein model is still questionable. Therefore, the severity classification is discussed controversially, pain monitoring is not standardized and analgesia is withheld frequently. In this project, we assess the severity in an acute pancreatitis model and aim to identify efficient analgesia not influencing the experiment's read-out.

Methods: We investigated an acute pancreatitis in male C57Bl/6 mice to detect pain and decreased wellbeing. Cerulein, a peptide commonly used to induce pancreatitis in rodents was injected intraperitoneal 12 times during two consecutive days. Mice injected with NaCl in the same time regime served as a control. We measured body weight, food and water intake; applied the burrowing test, mouse grimace scale and the von Frey test to detect pain and hypersensitivity in the abdomen. Fecal corticosterone metabolites were measured to detect short-term stress. Additionally, pancreases were histologically examined to grade inflammation. In further groups, we applied several analgesics (Paracetamol: Tramadol mixture, Metamizole, Buprenorphine) via the drinking water and tested them for efficacy, side effects and influence on the experimental model.

Results: Body weight change compared to baseline shows the highest decrease in Metamizole treated animals with slightly lower water intake in animals receiving Metamizole in the drinking water. Burrowing performance increases at the first measuring timepoint in all groups over time, at the second timepoint Metamizole treated animals do not

increase their performance like other groups. The MGS taken immediately after first Cerulein injections on day 1 and 2 shows higher values in Cerulein injected animals. There are no differences in the vonFrey test. The rear up frequency decreases over time. Parameters indicating abdominal pain are detected in all groups. Histological analysis reveales pancreatitis in all animals receiving Cerulein.

Conclusions: Experiments are currently still in progress and full results will be presented at the conference. Cerulein injections seem to be acutely painful. Preliminary results hint on a low level of pain from inflamed pancreases.

17h30 – 17h45

'One Europe': the challenge of consistency in severity classification

A. Zintzsch[1], A. Smith[2], N. Kostomitsopoulos[3], J.-B. Prins[4,5]

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The experience of recent years shows that severity classification is still insufficiently harmonised within the EU. Even among experts, animal procedures are often evaluated very differently. Consistent severity assessment and classification of procedures are essential for the ethical review process, and an indispensable part of planning, refining and evaluating animal experiments. Annual statistics of animals used in scientific research are intended to inform the public about the harms inflicted. Severity classification plays a crucial role in this process, and increased harmonisation should be sought.

As a first step, we have collected guidelines which have been published and are in use in Europe. We constructed an overview of classifications of experimental techniques and procedures, and for severity classification of genetically altered animals. The overview is available online at https://norecopa.no/severity. The overview serves as a valuable source for scientists, animal welfare bodies and representatives of the authorities. Severity classifications for specific procedures can be accessed at a glance. The overview will aid identification of areas where more research is needed, and will stimulate discussion so that additional information is submitted. This will hopefully lead to a second step: the refinement of the criteria used for severity classification, on the basis of better informed decisions.

Poster presentations - Refinement session

Poster 6

Shades of red: evaluating red light vision in laboratory rodents

N. Denk, S. Niklaus

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Introduction: Light conditions exert widespread effects on physiology and behavior. In order to enhance the ability to handle or observe laboratory rodents under nocturnal conditions, red light sources are used extensively in current laboratory research settings. This is based on the premise that there would be an absence of photoreception at red wavelengths by rodents. Several guidelines state that any light source transmitting >580 nm could be used appropriately in laboratory settings and would neither interfere with circadian rhythms nor interrupt nocturnal activities.

Methods: We evaluated the retinal response of mice and rats using a functional test (electroretinography) to evaluate a potential response of the retina to both a white light stimulus that was blocked with different red filter foils, as well as to a red LED light source of defined wavelengths (656+/-5nm). Albino and pigmented mice and rats were used in this study.

Results: Retinal response was significantly reduced when the light source was blocked with red filters but a retinal response was detected with all filters at certain light intensities. Absorbance spectra of the filters were variable, all of them transmitted some light also at a shorter wavelength spectrum. When examining the retinal response to red LED light of a more defined wavelength, our results clearly demonstrate that both retinas of all animals examined show a significant response to red light stimulation above a certain intensity. Even though significantly smaller compared to white light stimulation, the response amplitudes were, especially in albino rats, surprisingly high and reached values of the lower bandwidth of white light responses.

Conclusions: Our results demonstrate that the rodent retina does well react to red light which means that red light is, against the common assumption, not perceived as darkness by rodents but rather as medium to dim light. Considering this fact in research plans will ensure higher reproducibility of research results, especially experiments taking place in a reversed light cycle. Eliminating the variable light will ultimately increase the quality of research and animal housing conditions, contribute to a reduction of animal numbers and have a positive impact on Animal Welfare.

Poster 7

A rabbit model of chronic obstructive pulmonary disease: how to refine animal experiments in respiratory medicine

A. Dos Santos Rocha, R. Sudy, X. Belin, W. Habre

Dept of Acute Medicine - Unit for Anaesthesiological Investigations, Université de Genève, Genève, Switzerland

Introduction: Chronic obstructive pulmonary disease (COPD) is a leading cause of death worldwide. The development of new treatment strategies often require *in vivo* experiments, using appropriate COPD models. Such models involve invariably a chronic respiratory exposure to noxious substances, making animal welfare a primary concern. Hereafter, we propose a methodology to refine animal experiments in this context. We developed a rabbit model of COPD, aiming to test the benefits of a new mode of mechanical ventilation. We recently demonstrated the protective effects of physiological variable ventilation (PVV) in healthy lungs; in this work, we aimed at investigating whether PVV is beneficial in COPD.

Methods: COPD was induced in 16 New Zealand white rabbits, over 4 weeks, by nebulising microparticles of elastase and lipopolysaccharide in the lung. During this procedure, under sedation, the rabbits had a heating blanket, received artificial tears and breathe through a laryngeal mask. To refine the experiment, animals were evaluated twice a week using an original welfare score with nine parameters, as to early identify

signs of dyspnoea or suffering. If any of the score parameters were positive, rabbits would receive supplementary oxygen, bronchodilators and/or analgesic drugs. After COPD induction, the rabbits were randomised to receive 6h of mechanical ventilation with either variable ventilation (VV) or pressure-control mode (PC). Lung volumes, mechanics, histology and blood samples were assessed to evaluate the effects of ventilation.

Results: Animals in VV group showed significantly better oxygenation ratio (423 vs 341, $p< 0{,}001$), and less decrease in lung compliance (-34% vs -74%, $p=0{,}018$) after 6h of mechanical ventilation. Additionally, animals receiving VV had a lower lung clearance index (12.4 vs 17.7, $p=0.04$).

Conclusions: This methodological approach allowed us to recreate the features of COPD in the rabbit, while addressing animal wellbeing in all steps. Given the rabbit size and characteristics, allowing repeated measures, blood and tissue sampling in several timepoints, we were able to greatly reduce the number of animals used. Finally, we were able to find several advantages of physiological VV over conventional ventilation in the context of COPD.

Poster 9

Animal use and the 3Rs principles in ecology: European survey

M.A. Zemanova[1,2], S. Frey[2]

[1]University of Basel, Basel, [2]Animalfree Research, Bern, Switzerland

Introduction: Conservation of ecosystems and species gives rise to a paradox in which effective measures often involve very adverse (even lethal) interactions with several animal individuals on behalf of the good for the whole population. The 3Rs principles can provide scientists with guidelines on the ethical use of animals in research and education. However, the process of incorporating the 3Rs principles into wildlife research has been very slow and so far has been done on a rather small scale in comparison to animal research in laboratories.

Methods: In order to assess the attitudes and experience with animal use in ecological and wildlife research, as well as the awareness of the 3Rs principles, we launched an online questionnaire that was distributed to European ecologists.

Results: Surprisingly, less than half of the respondents were aware and could correctly list the 3Rs principles. Moreover, in their latest research, most respondents worked on vertebrates and yet only 17% of them used a non-invasive method; majority of the research animals were either killed, studied invasively or with combination of non-invasive and invasive methods. This survey also revealed that ecologists often feel ethical concerns regarding animal use in their work.

Conclusions: To our knowledge, this is the first attempt to assess the experience and attitudes towards animal use and the 3Rs principles among ecologists. The outcomes of this study should help improve further implementation of the 3Rs in ecological and wildlife research, and serve as a baseline for assessing success of such measures.

Poster 15

The danish 3R-Center

R.N. Nielsen

The Danish 3R-Center, The Danish Veterinary and Food Administration, Glostrup, Denmark

3R-News: The 3R-Center's website focuses on disseminating research and news relevant to persons interested in or working with 3R and laboratory animal science www.en.3rcenter.dk/

Research funding: Each year the 3R-Center funds 3R related research projects. Funded projects must be of a high scientific quality and relevance. Every year 1.5 million DKK is reserved for research funding.

3R-symposium: The annual international symposium is a significant event where around 200 researchers, veterinarians, animal caretakers, and other people with an interest in 3R meet and network. The program

is composed of Danish and international speakers and covers all three Rs. The 2019 Symposium will take place on November 12-13.

Teaching material: The 3R-Center has prepared teaching material for use in Danish lower and upper secondary classes. The material is free of charge.

Board members: Christine Nellemann (Chairman of the Board), Adrian Smith, Axel Kornerup Hansen, Erwin L. Roggen, Jan Lund Ottesen, Lisbeth E. Knudsen and Peter Bollen.

Poster 16

Gentle, efficient method for frequent blood sampling in group-housed rabbits on farms

M. Braconnier, S. Gebhardt-Henrich

Centre for Proper Housing: Poultry and Rabbits (ZTHZ) | Division of Animal Welfare |, VPH Institute University of Bern, Zollikofen, Switzerland

Introduction: Frequent blood sampling in rabbits ("Oryctolagus cuniculus") on farms is often associated with difficulties. Personnel expenses, as well as stress for the animals through handling, play a major role in the process. A stress- and pain-reduced, less invasive alternative is the blood collecting method by bugs ("Dipetalogaster maximus"). Through local anesthetic saliva, these animals are able to withdrawal blood unobtrusively and up to nine times their body weight.

Methods: In our study for hormone determination, blood was collected 6 times over 4 trials of 12 consecutive days each (n = 15). For this purpose, the rabbit was placed in a box and a container with a bug inside was attached to its chest. The bug was able to suck through the gauze at the bottom of the container.

Results: The average suction time was 17 minutes (min. 8 min; max. 43 min). Depending on the nymphal stage (N), an average of 0.5 ml (N3), 1.3 ml (N4) and 2.1 ml (N5) of blood was obtained. The blood was removed from the abdomen of the bugs using a 3ml syringe (G21 needle). A protein

in the bugs' saliva prevented it from clotting. Several animals could be sampled simultaneously by a single person using this method. The bugs could be reused after 5 weeks without feeding in some cases. There was no swelling or hematoma at the puncture site afterwards in any of the rabbits.

Conclusion: This procedure represents a gentle option for multiple blood sampling not only under laboratory conditions but also on farms.

Poster 19

Documentation of barbering in laboratory mice

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Introduction: Barbering, also known as hair trimming, whisker pulling or the Dalila effect, affects a significant proportion of mice kept in captivity[1]. Barbering can be either self-oriented or partner-oriented, but it does not seem to be a gesture of dominance[2]. While the occurrence of barbering is well known and even used as a factor to assess the welfare of laboratory mice[3], little is known about its prevention. Similarly little is known about the reasons leading to barbering. Hypotheses range from genetic predisposition and boredom to depression or anxiety.

Methods: Here we introduce a simple method to characterize barbering as a part of the daily routine within an animal facility. The categorization of barbering into different locations allows identifying if the barbering is self- or partner-oriented. Furthermore, potential changes over time such as spreading of the hairless area but also re-growth of hair can be documented.

Results: First evaluations of the collected data between April and September 2019 of our facilities show that less than 2% of mice are affected by barbering. In addition, females show a higher prevalence compared to males and most cases of barbering are partner-oriented.

Conclusions: For the future, we will use the collected data to identify potential risk factors of barbering such as cage types, breeding schemes, housing density or genetic background and to assess the effect of pilot projects to reduce the occurrence of barbering.

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Poster 34

The rule of three: optimal group sizes for housing male mice

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Aggression in male mice often leads to injury and death, making social housing difficult. We tested whether 1) small group sizes and 2) early age of allocation to a group decreases aggression and 3) manipulation increases aggression in male mice. A 14wk study was performed to assess the following conditions in male CD-1/ICR mice: group size (1, 2, or 3), age at grouping (5 or 7wks), and manipulation (daily scruffing or minimal weekly handling). Wounds, body weights, food consumption, nest scores, sucrose consumption, fecal corticosterone and hematology data were collected. At the end of the study, mice were euthanized and pelted to assess wounding with the pelt aggression lesion scale (PALS).

No signs of acute or chronic stress was observed in any of the groups. Trio housed mice showed less bite wounds than pairs. In general, animals in larger groups ate less but weighed most. High nest scores, increased sucrose and increased food consumption, and low body weights in individually housed mice suggest thermal stress, even when nesting material was provided. Based on this data CD-1 mice can successfully be housed for up to 14wks and groups of 3 may be the best for reducing even minor levels of aggression.

Poster 35

Exposure to low atmospheric pressure or nitrogen reduces locomotor activity and may increase anxiety in mice

A.R. Steiner[1], A. Renner[2], R. Bettschart-Wolfensberger[1], T.C. Gent[1]

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Introduction: Controlled atmospheric stunning (CAS) of mice is commonly performed with CO_2, however it is highly aversive and therefore alternatives are required. Proposed mechanisms of aversion to CO_2 are pain, air hunger and anxiety. Alternative CAS methods include oxygen displacement by inert gases or reduced atmospheric pressure (LAP). However, open questions remain over anxiety that may result from hypoxia.

Methods: Here we show, using elevated plus maze in mice, that exposure to nitrogen (N_2) or LAPS (causing O_2 partial pressures of 136.8 mmHg in groups N_2L and LAPSL, 114 mmHg in groups N_2M and LAPSM and 91.2 mmHg in groups N_2H and LAPSH), did not significantly increase anxiety compared to ambient conditions (control group). Animals exposed to CO_2 jumped off the maze prohibiting measurements. Experiments were video recorded and locomotor activity automatically tracked with Deeplabcut. One-way ANOVA was used for statistical analysis.

Results: We found that entries to open and closed arms did not significantly differ between groups. However, animals spent significantly less time on open than on closed arms when exposed to N_2M (45.7/- 20.8 vs. 122.8+/- 26.8 s, $p \leq 0.05$), LAPM (27.1 +/- 34.2 vs. 121.4 +/- 44.5 s, $p \leq 0.05$), N_2H (21.7+/- 16.9 vs. 138.3+/- 45.7 s, $p \leq 0.01$) and LAPH (30.7 +/- 46.5 vs. 132.8 +/- 94.7 s, $p \leq 0.01$), but not under ambient conditions (78.6 +/- 31.8 vs. 86.2 +/- 16.4 s). Average speed was significantly reduced compared to ambient conditions (5.4 +/- 0.9 cm/s) in LAPM (3.5 +/- 0.7 cm/s; $p \leq 0.05$), N_2H (2.3 +/- 0.5 cm/s; $p \leq 0.0001$) and LAPH (1.50 +/- 0.6 cm/s; $p \leq 0.0001$).

Conclusions: These data suggest that moderate levels of hypoxia reduce locomotor activity and may increase anxiety. However, the reduced activity may have resulted in prolonged resting times in the closed arms. In contrast, CO_2 caused significant aversion, preventing a direct comparison of anxiety with N_2 and LAPS. Further research is needed to clarify the degree of anxiety induced by hypoxia levels required for stunning and also whether overall anxiety is reduced compared to CO_2.

Poster 38

Does local infiltration of lidocaine-bupivacaine in combination with systemic analgesia provide superior pain relief for mouse laparotomy?

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Introduction: A multimodal pain management with local anesthesia (LA) added to systemic analgesia has the potential to decrease post-surgical pain perception and reduce hypersensitivity around the wound site. Researchers can find dosage and drug recommendations but not

systematic studies on the efficacy and side effects of LA in mouse surgery. The fear of overdosing or wound healing disorders is reported possibly resulting in LA not frequently being used in surgical mouse models. A refinement possibility could be missed.

Methods: We hypothesized that in a laparotomy mouse model, Lidocaine-Bupivacaine infiltration in combination with Paracetamol applied as sweet children's syrup (Dafalgan) via drinking water is superior to systemic analgesia of Paracetamol only. Four groups of C57Bl/6J mice were compared: Surgery with LA and Paracetamol, and surgery with LA or Paracetamol only. One group without surgery received anesthesia and Paracetamol only. Infiltration of Lidocaine-Bupivacaine was conducted by subcutaneous injection on the surgical site two minutes prior to incision. Body weight, food and water intake were measured. Nest complexity, activity, burrowing behavior and Mouse Grimace Scale were measured for detecting changes in animals' wellbeing and potential pain. Sugar consumption to detect anhedonia were analyzed. Additionally, animals were tested for mechanical hypersensitivity around the wound area with the von Frey test. Corticosterone metabolites in the feces were analyzed to depict short-term stress.

Results: Slight decrease of body weight, food and water intake after anesthesia and surgery is detected. Nest complexity is reduced after surgery or anesthesia. Female mice with LA only after surgery show the best burrowing performance. No differences between the groups are detected in sugar consumption, vonFrey test and the animal's activity. Lower values of FCM and MGS in animals treated with LA only after surgery.

Conclusions: The results show a sufficient ingestion of Paracetamol. No disorders in the wound healing or other side effects of LA treatment were detected. The Mouse Grimace Scale in all animals receiving Paracetamol were surprisingly high which led us to investigate the influence of Paracetamol on the used parameters. Full results will be presented at the conference.

Poster 39

Singing rate reflects animal welfare levels in isolated male zebra finches

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Songbirds, such as the zebra finch (Taeniopygia guttata), serve as the main animal model to study vocal learning — the ability to learn vocalization by imitation. In spite of several decades of research, ranging from behavior to molecular mechanisms, little is known about the impact of typical experimental procedures on the songbird's welfare. Ideally, the monitoring of stress and its effect on animal welfare should be stress-free. However, many existing methods in birds tend to be stressful and provide at best a measure of chronic stress. Here, we explore the possibility of using undirected singing (i.e. singing alone) as an indicator of animal welfare. In zebra finches, the undirected singing rate positively correlates with having a good body condition and with the success of attracting a female. Moreover, it is depressed under stressful conditions, such as under food and water deprivation and while wearing a heavy backpack.

We, therefore, tested this idea by performing a post-hoc analysis of more than three million songs in isolated male zebra finches, since isolation is usually a required condition to carry out vocal learning experiments. We found that most of the analyzed potential stressors temporarily decreased undirected singing rate. By contraposition, we inferred that a high-sustained undirected singing rate is compatible with high levels of animal welfare.

Since measuring the singing rate is contact-free and might reflect acute changes in stress levels, our findings show that the undirected singing rate could be a sensitive indicator of animal welfare, leading to refinement on current methods of welfare assessment in isolated male

zebra finches. Thus, we hope that this study contributes to extending welfare parameters for zebra finches used in research.

Poster 40

Challenging harms in the harm-benefit-analysis: managing uncertainty in GA welfare assessment - the case of harm assessment in genetically altered animals

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The use of animals in research requires careful ethical consideration of whether the burden on the animals is justified. As one important part of the project evaluation, a harm-benefit-analysis (HBA) has to be carried out in order to approve projects in line with EU Directive 2010/63/EU. This implies that harms and benefits of a particular project have to be assessed prospectively beforehand to weigh them afterwards. Although there are different models of weighing, it is clear that an assessment of prospective harms and benefits is a precondition for any weighing procedure.

In this context, projects for the generation of new genetically altered (GA) lines or breeding and maintenance of GA animals that are likely to develop a harmful phenotype raise new issues. The unique feature of new GA lines is that a significant lack of knowledge makes it difficult and logically impossible to estimate harms prospectively with sufficient certainty since it is not predictable what sort of harm - if at all - the animals are going to experience. Therefore, this contribution aims at dealing with the new challenges of harm assessment in GA animals and its implications on the HBA. A practical guideline is under development that will serve as an overview and guidance for relevant harm factors and addresses the main challenges, e.g. dealing with uncertainties and pointing out the consequences in the process of HBA.

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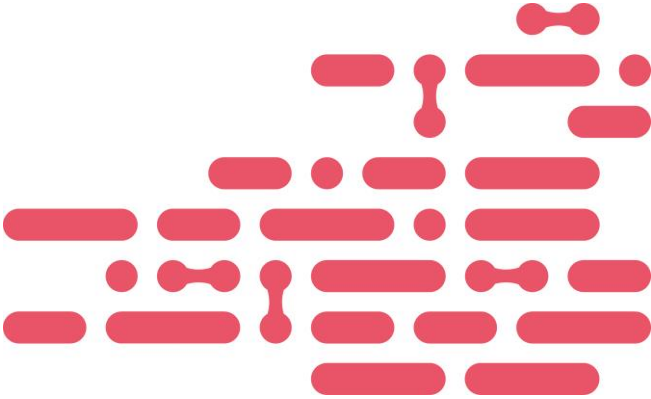

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