Abstracts of the 10th World Congress, Seattle, 2017

Volume 6, No. 1
ISSN 2194-0479
(2017)

ALTEX Proceedings

Springer Spektrum

WELCOME

Dear WC10 participants,

Thank you all for coming to the 10th World Congress on Alternatives and Animals in the Life Sciences in Seattle, Washington, USA. This is the first WC in the US since 2002 in New Orleans. We heartily welcome you to this Pacific Northwest city. Seattle located on Puget Sound, offers beautiful mountain and seawater vistas, high tech giants and recreational opportunities both in town and in our three surrounding national parks.

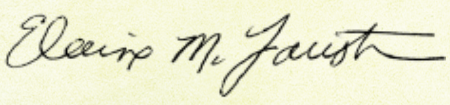
WC10 is a milestone in many ways. It is the tenth in a series of Congresses that had its beginnings in Baltimore in 1993, when Alan Goldberg and Bert van Zutphen had the foresight to highlight progress in the field of the 3Rs in an international forum. WC10 also marks the 10th anniversary of the seminal National Academies report – *Toxicity Testing in the 21st Century – A Vision and a Strategy* – that began the hard core revolution to transform toxicity testing with the goal of eliminating the vast majority of animals and focusing on pathway analysis through human cell cultures, computer modeling and data mining. This Congress also highlights current developments in new cell and 3-D organoid culture methods as well as organs-on-a-chip technologies. Other sessions focus on the refinement of methods that continue to use animals by advances in pain assessment and alleviation, animal husbandry and environmental enrichment, and prominent behavioral interventions to improve animal welfare. The Congress also illustrates the progress in educating scientists and the public about the 3Rs as well as addressing more sophisticated and serious ethical questions surrounding the use of animals in research, testing and education. For this Congress, we have chosen the theme:

The Three Rs in Action

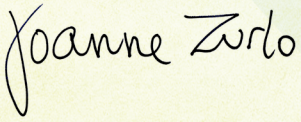
to highlight the extraordinary number of advances in developing and implementing the 3Rs into cutting edge, innovative science. We have had a remarkable response to our call for abstracts, receiving over 800 abstracts. We are proud to host 68 platform sessions covering nine themes and over 350 posters. Our plenary speakers highlight the significance of the Tox 21 initiative, the advent of application of big data to the 3Rs and how the 3Rs have influenced the pharmaceutical industry. We are pleased to have been able to support the attendance of students to present their work in platform or poster sessions.

In addition to the scientific program, we hope that the social program reflecting the culture of Seattle and picturesque and friendly atmosphere in combination with world-class cuisine, wine and music will further stimulate the exchange of scientific knowledge and expertise. As an added bonus, we are especially pleased to be able to offer you a glimpse of the solar eclipse that will occur on Monday, August 21.

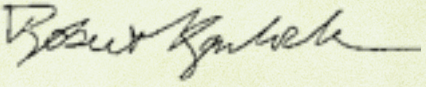
We wish to thank the very high number of sponsors and exhibitors, since without their support we would not be able to ensure the high scientific standard of WC10, all participants for their contributions, the Scientific Committee, Theme Leads, local organizing committee and the management team at FASEB for their continuous support in planning and organizing WC10 in Seattle.



Elaine M. Faustman, PhD, DABT
Chair, WC 10
University of Washington
Seattle, WA



Joanne Zurlo, PhD
Co-Chair, WC 10
Johns Hopkins University
Baltimore, MD



Robert Kavlock, PhD
Co-Chair, WC 10
USEPA
Washington, DC

Dear WC10 participants,

ALTEX Proceedings is honored to publish the Abstract book of the 10th World Congress on Alternatives and Animal Use in the Life Sciences in Seattle, WA.

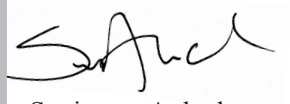
We are publishing the Abstract book online ahead of the congress to allow you to plan your participation in the different sessions ahead of your journey to the Pacific Northwest. Note that no printed copies of the Abstract book will be distributed at the congress to reduce the environmental impact and to avoid weighing down your luggage. The full Abstract book is available at http://www.altex.ch/altex-proceedings.

This Abstract book contains short summaries of almost 700 abstracts that were accepted for oral or poster presentations. These are shared over the 9 Congress Themes covering ethics, lessons learned, innovative models for safety and efficacy, sustainability, systems biology and big data, 3Rs in academia, translation, refinement and animal welfare and global cooperation. The abstracts represent the work of contributing authors from a total of 43 countries from six continents.

We thank Michele McDermott and Bridget van Egmond from FASEB and James Gentry from MiraSmart Conferencing for their cooperation in producing the Abstract book. We are grateful to the Doerenkamp-Zbinden Foundation, Switzerland for generously funding the production of the Abstract book.

We wish all participants of WC10 that the congress will inform, inspire and connect you to drive forward your work on alternatives to animal experimentation. Please note that WC11 will be held in Maastricht in 2020.

With best wishes,



Sonja von Aulock

Editor in chief, ALTEX & ALTEX Proceedings

Theme VI – 3Rs in Academia

Theme VII – Translation

Theme VIII – Refinement and Animal Welfare

Theme IX – Global Cooperation

Plenary Lectures

PLEN-390

The changing role of the 3Rs in the pharmaceutical industry

Margaret Landi
Office of Animal Welfare, Ethics and Strategy, GSK Pharmaceuticals, King of Prussia, United States
margaret.s.landi@gsk.com

The 3Rs is well established as an iconic paradigm for review and debate of studies requiring animals. In the pharmaceutical industry animal studies are divided into those looking for targets and leads for efficacy (pharmacology) and those designed for toxicity and safety (drug testing). In addition animals are still required for quality control and batch release for vaccines and biological products. Recently studies using animals in pharmaceutical companies has expanded to device work; such as in bioelectrical modulation for disease treatment and/or prevention. This plenary presentation will review not only present work in the 3Rs, but also future opportunities through both aspirational and realistic lenses.

PLEN-624

Predictive evaluation of environmental impact driving sustainable innovation in the cosmetic industry

Jacques Leclaire
L'Oréal, Clichy, France
jleclaire@rd.loreal.com

Nowadays, cosmetics and personal care products (CPCP) are most frequently used in the bathroom. Used rinsed off products flow down the drain where they mix with wastewaters. In industrialized countries they are directed to sewage treatment plants, but in many developing countries wastewaters may directly be discharged into rivers or the sea shore. In addition, certain products such as sunscreens may be directly released by swimmers in lakes, rivers or the ocean. Assessing their potential environmental impact faces significant methodology challenges because of their extremely diverse composition, from single ingredients to heterogeneous complex mixtures. Predicting and anticipating the impact of CPCP on the environment, at the end of life or for their production, early in the research process is a key element to innovate sustainably. The purpose of this presentation will be to present the challenges, trends and advances in these fields but also contributions to the innovation process.

PLEN-733

Using 21st century science for risk-related evaluations: An overview of the report

Jonathan Samet
University of Southern California, CA, United States
jsamet@med.usc.edu

The report Using 21st Century Science for Risk-Related Evaluations, released by the National Academies of Sciences, Engineering, and Medicine in January 2017 and was a follow-on to the 2007 report Toxicity Testing in the 21st Century: A Vision and a Strategy and the 2012 report Exposure Science in the 21st Century: A Vision and a Strategy. The 2017 report describes advances in toxicology, exposure science and epidemiology since publication of the earlier reports and focused on practical applications of the data being generated using 21st century science and approaches. Case studies are provided in several appendixes, but the report emphasizes that the ability to generate the data has outpaced the ability to use the data for risk assessment and decision making and greater attention needs to be paid to data interpretation and integration. This presentation introduces the 21st Century Science report and its principal findings. While the report indicates ways to immediately apply 21st Century methods to risk evaluation issues, it also describes a path forward on some of the remaining and unsolved challenges in doing so.

Reference

Link to report: https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations

PLEN-736

Rat helping behavior: Implications for husbandry

Peggy Mason

Neurobiology, University of Chicago, Chicago, IL, United States
pmason@uchicago.edu

United States Enrichment programs aim to provide rodents with a complex physical environment and access to an exercise wheel. Social enrichment has received far less attention. Yet, rats are motivated by other rats to take actions that do not occur in response to physical objects. Using a paradigm in which rats are given the opportunity to release a cagemate trapped within a restrainer, we have learned a great deal about rats' attitudes toward other rats in distress. The motivation to help is independent of immediate social reward as rats release trapped rats into spaces that they (the helper) cannot access. Rats treated with an anxiolytic do not help a trapped cagemate, suggesting that it is affect that motivates the helper to actively help. Yet, to our surprise, unpublished results suggest that rats help even when the trapped rat shows no distress, suggesting that cognitive empathy can motivate a rat to help. Cognitive empathy refers to the communication and understanding of another's experience through modes that do not involve affect. In sum, rats show behaviors that resemble not only emotional empathy but also cognitive empathy, heretofore thought to be the exclusive domain of primates, particularly humans.

Theme I – Ethics

Coordinators
Adam Shriver, University of Pennsylvania, Philadelphia, PA, United States
Catherine E. Willett, The Humane Society of the United States, Washington, DC, United States
Dan Weary, Department of Animal Welfare Program, Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Canada
Kathrin Herrman, Free University of Berlin, Berlin, Germany

Oral Presentations

Session I-1: Harm-Benefit and Beyond

Co-Chairs
Jeff Sebo, New York University, New York, NY, United States
Judy MacArthur Clark, JMC Consultancy, Sandwich, United Kingdom

I-1-750

Narrow and wide harm-benefit analysis

Jeff Sebo
New York University, New York, NY, United States
jeffsebo@gmail.com

In this talk I make a distinction between what I call narrow and wide harm-benefit analysis, and I explore the implications of this distinction for animal research. I begin by summarizing what I call narrow harm-benefit analysis, which focuses on the relatively direct effects of animal research. These effects include animal suffering and scientific setbacks due to false positives and negatives, as well as human happiness and scientific progress due to true positives and negatives. I then summarize what I call wide harm-benefit analysis, which focuses on direct as well as indirect effects of animal research. These effects include the impact that animal research has on medical research more generally, as well as the impact that it has on our relationships with and treatment of nonhuman animals more generally. I conclude that wide harm-benefit analysis likely tells against animal research more decisively than narrow harm-benefit analysis does, and that wide harm-benefit analysis should be informing our activism, advocacy, and politics around this issue whether or not it should also be informing our institutional review discussions around this issue.

I-1-663

Harm benefit analysis: The UK experience 1986-2017

Kate Chandler
Animals in Science Regulation Unit, UK Home Office, United Kingdom
Kate.Chandler@homeoffice.gsi.gov.uk

The UK Inspectorate, part of the Animals in Science Regulation Unit (ASRU) of the UK Government, has undertaken harm-benefit analysis (HBA) as the cornerstone of project licence evaluation since 1986. HBA is required by law, under the Animals (Scientific Procedures) Act. Views on evaluation of harms and benefit continue to evolve, and are informed by scientific evidence and societal concerns.

Where projects raise issues requiring more detailed consideration (e.g. matters of particular public concern), additional advice may be sought by Inspectors or the Secretary of State. Applications may be referred within the Inspectorate, to officials or Ministers, to our independent advisory committee (the Animals in Science Committee: the ASC), and/or to external subject-expert assessors. Since the transposition of 2010/63/EU, we have published detailed Guidance on how HBA is undertaken in the UK.

Reference

https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/487914/Harm_Benefit_Analysis__2_.pdf

I-1-388

Harm-benefit analysis – How can and should it contribute to the assessment of animal experiments?

Peter Sandøe[1], Herwig Grimm[2] and I. Anna S. Olsson[3]
[1]Dept. of Food and Resource Economics and Dept. of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]Messerli Research Institute, University of Veterinary Medicine, Vienna, Austria; [3]Laboratory Animal Science, i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal
pes@sund.ku.dk

That an animal experiment can be justified by a favourable balance between harms to the animals directly affected, and likely benefits to humans, is the rationale behind much current legislation requiring that experiments comply with the 3Rs and serve certain specified purposes. However, there has been a movement towards adding so-called harm-benefit analysis as an *additional* requirement in assessing experiments. Here we analyse and discuss the content and role of such a requirement. Either the requirement is understood as imposing *a metric* that, assuming that harms and benefits fit on the same scale, will generate absolute and rather restrictive verdicts, or as a *checklist* the aim of which is to ensure that the final assessment happens on a properly informed basis. We argue that a system based on the first interpretation is unfeasible, whereas a system based on the second interpretation may serve as an important addition to the 3Rs in ensuring sound assessments of animal experiments.

I-1-434

Can harm-benefit analyses mature into less structurally biased concepts to meet societal demands for replacement?

Charlotte Blattner

Department of Philosophy, Queen's University, Kingston, Ontario, Canada

ch.blattner@gmx.ch

The number of animals used for experimental purposes worldwide is now the same as it was in the 1980s, even though society demands that more should be done to replace animals in research. Debates about the continuing high use of animals in research are regularly held at the expense of scientists. This article argues that the failure is primarily a *regulatory* one, caused by a misdesign of harm-benefit analyses.

To meet societal claims for better animal protection, I explore the 3Rs' potential to mature into a more viable concept for the future of animal law. I first suggest a reverse hierarchical understanding of the 3Rs on the basis of the legal interpretation and the proportionality and precautionary principles. Second, I propose a balance of interests in qualitative terms, which is increasingly demanded by animal law experts. Third, I explore whether rights to life and bodily and mental integrity of animals would render cost-benefit analyses less structurally biased and whether they would be feasible from a political perspective.

References

Bailey, J., Thew, M. and Balls, M. (2014). An analysis of the use of animal models in predicting human toxicology and drug safety. *Altern Lab Anim 42*, 181-199.

Baker, M. (2016). 1,500 scientists lift the lid on reproducibility. *Nature 533*, 452-454.

Clemence, M. and Leaman, J. (2016). *Public Attitudes to Animal Research in 2016*. London: Ipsos Mori.

Curnutt, J. (2011). Animals and the Law: Contemporary Legal Issues. ABC-CLIO, Santa Barbara.

Ferrari, A. and Gerritsen, V. (2015). Güterabwägung. In A. Ferrari and K. Petrus (eds.), *Lexikon der Mensch-Tier Beziehungen,* 139-143. Bielefeld: Transcript.

Funk, C. and Rainie, L. (2015). *Public and Scientists' View on Science and Society, c. 4 Attitudes and Beliefs on Science and Technology Topics*. Washington, D.C.: Pew Research Center.

Gerritsen, V. (2015). Evaluation process for animal experiment applications in Switzerland. *ALTEX Proc 4*, 37-40.

Greek, R. and Menache, A. (2013). Systematic reviews of animal models: Methodology vs epistemology. *Int J Med Sci 10*, 206-221.

Javier Guillén (ed.), *Laboratory Animals: Regulations and Recommendations for Global Collaborative Research* (61-93). San Diego, London, Waltham: Academic Press.

Kuhlau, F., Höglund, A. T., Evers, K. and Eriksson, S. (2011). A precautionary principle for dual use research in the life sciences. *Bioethics 25*, 1-8.

McCausland, C. (2014). The five freedoms of animal welfare are rights. *J Agric Environ Ethics 27*, 649-662.

Peters, A. (2012). *Rechtsgutachten zu verschiedenen Fragen im Zusammenhang mit der EU-Tierversuchsrichtlinie, insb. Zur Unionsrechts- und Verfassungskonformität des Entwurfs eines Dritten Gesetzes zur Änderung des Tierschutzgesetzes sowie des Entwurfs einer Verordnung zur Umsetzung der Richtlinie 2010/63/EU*. Zürich: Schulthess, Schriften zum Tier im Recht.

Peters, A. (2016). Liberté, égalité, animalité: Human-animal comparisons in law. *TEL 5*, 25-53.

Robertson, I. A. (2015). *Animals, Welfare and the Law: Fundamental Principles for Critical Assessment*. Oxon: Routledge.

Taylor, K., Gordon, N., Langley, G. and Higgins, W. (2008). Estimates for worldwide laboratory animal use in 2005. *Altern Lab Anim 36*, 327-342.

Taylor, K. (2013). Animals in Research. In A. Linzey (ed.), *The Global Guide to Animal Protection* (178-180). Urbana, Chicago, Springfield: University of Illinois Press.

Taylor, K. and Rego, L. (2016). EU statistics on animal experiments for 2014. *ALTEX 33*, 465-468.

I-1-625

The potential role of assessing study quality in harm-benefit analysis

Emily Sena

University of Edinburgh, Edinburgh, United Kingdom

sena.emily@gmail.com

A harm-benefit analysis (HBA) seeks to quantify the potential benefit of a research project and determines whether this outweighs likely harm to animals. Currently, this decision-making process does not take into account measures by researchers to reduce the introduction of potential sources of bias in their experimental design. An important factor in the rigorous design of an experiment is ensuring the internal validity of an experiment; that is to instil confidence in the cause-effect relationship by actions such as randomisation and blinding. Systematic review of the *in vivo* literature suggests that only about a third of studies report taking these measures to reduce the risk of bias. Studies at high risk of bias are associated with reporting inflated effects that likely contributes to the reproducibility crisis.

If the purpose of a HBA is to quantify potential benefit, assessment of the experimental design and the measures taken to reduce risks of bias are pertinent to assessing the likelihood of a study realising its potential benefit.

I-1-821

Harm benefit analysis (HBA): Making good decisions

Judy MacArthur Clark

JMC Consultancy, Sandwich, United Kingdom

judymacarthurclark@gmail.com

Exercising a harm-benefit analysis (HBA) requires an individual or group to make a decision based upon an apparent systematic approach to estimating the strengths and weaknesses of alternative options. However, this calculation is surrounded by much uncertainty, not least the difficulty in assessing potential benefit as well as potential harms.

Furthermore, individuals operating independently may reach decisions based largely on prior experience whereas those deciding in a group will tend to be influenced either by "groupthink" or by the strength of views expressed by different members. In both cases, "paralysis by analysis" can prevail so that a decision is very protracted or never taken leading to frustration on all sides.

In this paper, I will critically consider different models by which committees and individuals address HBA decision making. There may be no perfect method – but awareness of the strengths, weaknesses and pitfalls of diverse approaches may help to improve outcomes.

As session co-chair, I will also summarise key points made in the earlier papers and related posters leading into a guided discussion.

Session I-2: How Can IACUCs and Ethics Committees Improve to Further Benefit Animals

Co-Chairs
Larry Carbone, University of California San Francisco, San Francisco, CA, United States
Jeffrey Kahn, Johns Hopkins Berman Institute of Bioethics, Baltimore, MD, United States

I-2-809

Ethical evaluation of animal experiments: Mandate and lessons from the past

Roman Kolar, Kristina Wagner and Irmela Ruhdel
Animal Welfare Academy, German Animal Welfare Federation, Neubiberg, Germany
roman.kolar@tierschutzakademie.de

The regulation of animal experiments has a long history. Its origins stem from public debate about the question when and to which extent animal suffering can be justified in the context of research, testing, and education. That public discourse has led to various legislative acts that have aimed at providing for a regulatory framework for the practical performance of animal experiments, and the underlying theoretical, including ethical, considerations. Some of the systems in place have been functioning for decades, others are relatively new. Some have proved to function properly concerning specific aspects whereas others have not. The analysis of the problems that have been encountered can and should serve as a basis for the way forward. This refers mainly to a common understanding of the issues and the process with regard to the cost-benefit analysis, i.e. weighing animal suffering against the supposed benefit. Concerns raised in this context also include the lacking expert knowledge on ethics, the 3Rs or the specific scientific problems discussed in individual ethics committees, the insufficient existing approaches for regulatory demanded tests, and others.

I-2-556

A systematic retrospective assessment of the harms and benefits of pre-clinical animal research for 6 interventions

Pandora Pound and Christine Nicol
School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom
pandora.pound@bristol.ac.uk

Research using animals in the EU is subjected to a harm benefit assessment (HBA) whereby the anticipated benefits of the research are weighed against its predicted harms to animals (EU, 2010). We conducted a retrospective HBA to assess whether harms to animals were justified by benefits to humans.

A sample of pre-clinical animal studies (n = 228) relating to 6 interventions whose clinical relevance had already been determined was identified (Perel et al., 2007). Data on harms were extracted and categorised for severity by a panel of veterinary experts using the EU classification (EU, 2010).

Two interventions were potentially beneficial to humans, 1 was potentially partially beneficial and 3 were not beneficial. For all 6 interventions, scientific rigour was poor and harms were generally categorised as moderate to severe. Ninety seven percent of studies did not report analgesia use and 13% did not report anaesthesia use. Endpoints ranged from hours to 2 years with 33% of studies reporting deaths before endpoint. Seventy percent of studies did not report any welfare information and only 1% reported post-operative care.

Many animals suffered severe harms that could not be justified in terms of human benefit.

References

EU (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J Eur Union L276*, 33-79.

Perel, P., Roberts, I., Sena, E. et al. (2007). Comparison of treatment effects between animal experiments and clinical trials: Systematic review. *BMJ 334*, 197.

I-2-807

Unalleviated pain and distress: How can ethics committees and IACUCs best evaluate the justification?

Larry Carbone
Inst. Animal Care and Use Program, University of California San Francisco, San Francisco, CA, United States
larry.carbone@ucsf.edu

Laboratory animal regulations and guidelines in most countries call for managing, preventing and minimizing animal pain and distress, so far as analgesics and other refinements will not interfere with the quality of the data. Since the early 1970s, the United States *Animal Welfare Act* has recognized this departure from maximizing animal welfare, requiring animals on such protocols to be listed separately in Column E of the facility's annual report. This presentation will discuss the challenges for researchers, veterinarians and oversight committees in deciding when analgesics and other refinements might lower the quality of the data. It covers the need to further recognize what unalleviated pain and distress might do to the data, and to weigh the relative impacts of pain and pain management, and the unfortunate reality that little of the necessary scientific information for most models is established and published.

References

Carbone, L. (2012). Pain management standards in the eighth edition of the guide for the care and use of laboratory animals. *JAALAS 51*, 322-328

Carbone, L. and Austrin, J. (2016). Pain and laboratory animals: Publication practices for better data reproducibility and better animal welfare. *PLoS One 11*, e0155001.

I-2-808

IACUC review of the justification of proposed research – What can be learned from IRBs?

Jeffrey Kahn

Johns Hopkins Berman Institute of Bioethics, Baltimore, MD, United States
jeffkahn@jhu.edu

As in the review of proposed research involving human subjects by Institutional Review Boards (IRBs), the prospective review of animal research by IACUCs must assess whether regulatory requirements are met, intended to protect research subjects and assure that research is ethically performed. The extent to which scientific justification of research can and should be considered in prospective review by IRBs and IACUCs is a point of debate.

This presentation will discuss arguments about whether scientific review should be included in ethics oversight of research on human or animal subjects, what the IACUC community can learn from experience on the part of IRBs, and will explore what the application of a necessity framework has to offer to the debate.

Session I-3: Ethical Selection of Species – Round Table

Chair
Andrew Rowan, Humane Society International, Gaithersburg, MD, United States

I-3-753

Ethical consideration of the use of different species in experimentation

Jeffrey Kahn[1], Peter Sandøe[2] and Dan Weary[3]

[1]Johns Hopkins Berman Institute of Bioethics, Baltimore, MD, United States; [2]Dept. of Food and Resource Economics and Dept. of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark; [3]Department of Animal Welfare Program, Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada
jeffkahn@jhu.edu

Public support for animal research in the United States has been declining slowly but steadily since the 1950s. Various surveys around the globe have demonstrated similar trends and also indicate that the level of public support for animal research varies substantially (10 percentage points or more in some surveys) by the type of animal (e.g. mouse, dog or primate) that the question specifies and even more dramatically by the level of suffering the animals are perceived to experience. In general, there is less public opposition to the use of mice than to the use of dogs or primates and less opposition where the research is viewed as causing little harm to the animals. These issue (the ethics of using different types of animals in research and testing and the different perceptions of harm and benefit by members of the public and by scientists) that are intended to be the focus of the round-table discussion. While public concern tends to focus on the level of perceived harm to the animals, research scientists tend to emphasize the expected benefit to humans. The session will be chaired by Andrew Rowan who will provide a brief introductory overview. The three panelists will then set up some of the ethical issues relating to primates (Jeffrey Kahn), other animals used in research (Daniel Weary) and rodents versus dogs (Peter Sandoe) in short presentations. Others present at the Round Table will then be invited to comment on these three presentations and to introduce new arguments and ideas that may not have been raised already. It is anticipated that there will be many who will want to participate so presenters (7 minutes) and audience participants (3 minutes) will be strictly limited as to the amount of time that they will have to speak.

Session I-4: Beyond Refinement – Can We Provide Animals Used in Research with a Good Life Experience

Co-Chairs
Kathrin Herrmann, Free University Berlin, Berlin, Germany
Adam Shriver, University of Pennsylvania, Philadelphia, PA, United States

I-4-755

A suffering-centric account of the importance of positive emotions

Adam Shriver

Animal Welfare Program and Centre for Applied Ethics, University of British Columbia, Vancouver, BC, Canada
ashriver@mail.med.upenn.edu

It is often suggested that ethical perspectives prioritizing the disvalue of suffering are at odds with approaches that emphasize positive states of welfare. In this presentation, I will argue that even on ethical approaches that hold that the prevention of suffering should be the most important aim of welfare protections, the promotion of positive emotions is critically important. I present two primary forms of evidence in favor of this conclusion. First, I review the literature on the role of anhedonia in humans, where a lack of the capacity to feel positive emotions leads to an intensely negative subjective experience. Second, I highlight a wide range of research from the neurosciences that demonstrates that feelings of pleasure have an inhibitory effect on neural pathways involved in pain and anxiety. From this evidence, it follows that the facilitation of positive emotions is one of the best ways to minimize suffering in laboratory animals, and perhaps is a necessary condition for a true absence of suffering. As such, the need to ensure positive states of welfare through enrichment is not dependent upon any particular commitment about how to weigh positive emotions against negative emotions, but instead should be regarded as a priority for all.

I-4-623

Taking animals used in research seriously: At a crossroads between ethics and animal welfare

Walter Sanchez-Suarez

School of Psychology/Sackler Centre for Consciousness Science, University of Sussex, Villamayor, Spain
w.sanchez-suarez@sussex.ac.uk

It is not possible to come up with an accurate estimation of the number of nonhuman animals used in research worldwide, but – even when not taking invertebrates into account – most sources (e.g., RSPCA) agree with overwhelming figures above 100 million animals per year. Importantly, many of these research practices involve invasive procedures. Within this context, the label "ethical" is often used to describe these practices; but do they actually qualify as ethical from a normative viewpoint? And are we taking advantage of the words "ethical" and "humane" in order to prevent society from grasping a real notion of what we are doing to these animals? I will first focus on these questions by evaluating whether research involving animals in general, and invasive procedures in particular, can be regarded as ethical according the most relevant ethical theories. Finally, even if some of these practices were to qualify as ethical, could they be compatible with providing those animals involved with good welfare? In order to elucidate whether this is the case, the second part of the talk will deal with this question in the light of the most thorough concepts of animal welfare.

Reference

https://www.rspca.org.uk/es/adviceandwelfare/laboratory

I-4-455

Is refinement enough to promote psychological well-being?

Stacy Lopresti-Goodman

Psychology, Marymount University, Arlington, VA, United States
stacy.lopresti-goodman@marymount.edu

Refinement is aimed at improving the welfare of animals in laboratories by modifying experimental procedures and husbandry practices to minimize pain and distress (Russell and Burch, 1959; Fenwick et al., 2009). Despite the goal of yielding more humane science, animals in laboratories still experience negative welfare. This talk will focus on behavioral abnormalities and psychological distress resulting from standard laboratory practices (Lopresti-Goodman, 2016; Chandna et al., 2015). It will also highlight persistent problems that have been documented in animals who have been released from laboratories and retired to sanctuaries (e.g. chimpanzees) (Lopresti-Goodman, 2015), or adopted into private homes (e.g. dogs) (Lopresti-Goodman, submitted).

References

Chandna, A., Niebo, M., Lopresti-Goodman, S. M. and Goodman, J. R. (2015). Single housing of primates in U.S. laboratories: A growing problem with shrinking transparency. *Altern Lab Anim 43*, 30-33.

Fenwick, N., Griffin, G. and Gauthier, C. (2009). The welfare of animals used in science: How the "Three Rs" ethic guides improvements. *Can Vet J 50*, 523-530.

Lopresti-Goodman, S. M., Bezner, J. and Ritter, C. (2015). Psychological distress in chimpanzees rescued from laboratories. *J Trauma Dissociation 16*, 349-366.

Lopresti-Goodman, S. M. (2016). A review of psychological distress in laboratory living primates. Paper given as part of symposium, Use and care of captive non-human primates: Evaluating and improving ethical requirements. Joint Meeting of the International Primatological Society and American Society of Primatologist, Chicago, IL.

Lopresti-Goodman, S. M. and Bensmiller, N. (submitted). A comparison of ex-laboratory dogs' psychological and behavioral characteristics compared to non-laboratory dogs.

Russell, W. M. S. and Burch, R. L. (1959). *The Principles of Humane Experimental Technique*. London, UK: Universities Federation for Animal Welfare.

I-4-520

A better life for research animals by fostering a culture of compassion amongst researchers

Catherine Schuppli[1], Andrea Walterhouse[2], Vivian Chew[2], Nevene Hammoud[2], Lara Kolody[2], Bee-Li Tan[2], Joanna Makowska[2], Sarah Mcnamara[2], Joyce Sato-Reinhold[2], Venessa Wong[2] and Daniel Weary[2]

[1]Animal Welfare Program, and Animal Care Services, University of British Columbia, Vancouver, BC, Canada; [2]Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada
cathy.schuppli@ubc.ca

This study used a novel educational intervention to test if exposure to socialized rats that demonstrated complex mental and behavioural abilities, promoted compassion amongst animal researchers. Six rats were trained using positive reinforcement techniques to participate in tasks such as jumping onto a scale, entering a restraining tube, and fetching and lifting objects. Researchers who enrolled in a rodent handling course observed these rats perform and handled them for practice. After the class, researchers (17) participated in focus groups (5) to discuss their impressions of the rats. Using qualitative analyses key findings include that all participants were "amazed" by the rat's performance and the personal relationship with their handlers. All believed socializing rats with humans reduced stress but views differed on the potential effects on data. There was concern about the emotional burden on researchers of "sacrificing" their subjects after developing similar relationships.

I-4-699

Thinking "inside" the box: Housing essential for a good life

Joanna Makowska

Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada
inez.joanna.makowska@gmail.com

Housing is the most pervasive element of research animals' lives, largely dictating the limits of their behaviour and experiences. Conventional laboratory cages do not allow rodents to perform many natural behaviours. When rats were housed in semi-naturalistic cages, they burrowed, climbed, and stood upright approximately 30, 75 and 180 times per day, respectively. Control standard-housed rats, unable to perform these behaviours, performed nine times more lateral stretches than rats in the semi-naturalistic cages, likely to alleviate stiffness caused by low mobility. A follow-up study on anticipatory behaviour indicated that these standard-housed rats were experiencing poorer welfare than the semi-naturalistic-housed rats. A study on housing mice in three interconnected cages found that mice prefer to nest far from where they eliminate, and cover their waste with spare bedding. These studies show how providing research animals with appropriate, species-specific housing that allows for natural behaviors is important to good welfare.

I-4-387

Refinement and the culture of care: What is in it for the animals?

Kathrin Herrmann

Dahlem Research School for Biomedical Sciences, Free University Berlin, Berlin, Germany
Kathrin.Herrmann@fu-berlin.de

As a consequence of changes in legislation and increased public discontent about the use of animals for experimentation, Refinement, the 3rd R, has taken center stage in the laboratory animal science community in recent years.

The first large-scale retrospective assessment of research applications was carried out, which involved recovery surgical procedures with rats and mice, to evaluate practices in anesthesia, analgesia, post-operative monitoring, humane endpoints and killing methods in Germany before the implementation of Directive 2010/63/EU for the protection of animals used for scientific purposes. Over 500 applications from 2010 were reviewed. The goal was to determine if legal requirements regarding Refinement were met or overfulfilled, as a true culture of care would give reason to expect.

In this paper, the principal findings will be presented and discussed and recommendations for best practice approaches will be given. Furthermore, the question will be posed whether an optimal culture of care could ever provide a good life for animals who live in a laboratory environment and are used in invasive experiments.

References

Brown, M. (2014). Creating a culture of care. NC3Rs News & Blog. https://www.nc3rs.org.uk/news/creating-culture-care.

Clemence, M. and Leaman, J. (2016). *Public Attitudes to Animal Research in 2016*. London: Ipsos Mori.

Flecknell, P., Leach, M. and Bateson, M. (2011). Affective state and quality of life in mice. *Pain 152*, 963-964.

Jirkof, P., Cesarovic, N., Rettich, A. et al. (2010). Burrowing behavior as an indicator of post-laparotomy pain in mice. *Front Behav Neurosci 4*, 165.

Makowska, I. and Weary, D. (2016). The importance of burrowing, climbing and standing upright for laboratory rats. *R Soc Open Sci 3*, 160136.

Miller, A., Flecknell, P., Leach, M. and Roughan, J. (2011). A comparison of a manual and an automated behavioural analysis method for assessing post-operative pain in mice. *Appl Anim Behav Sci 131*, 138-144.

Russell, W. and Burch, R. (1959). *The Principles of Humane Experimental Technique*. 1st edition. London: Methuen & Co Ltd.

Session I-5: Ethical Considerations for New Technologies Using Animals – Round Table

Chair
Adam Shriver, University of Pennsylvania, Philadelphia, PA, United States

I-5-746

Chimera troubles: On a better ethics of human-animal chimera research

Andrew Fenton
Philosophy, Dalhousie University, Halifax, NS, Canada
atf@dal.ca

The NIH is changing its guidelines on funding studies of human-animal chimeras. I will highlight several critical bioethical issues in chimera research with a particular eye to the kind of animal studies the NIH is interested in funding. I argue that (i) the creation of these human-animal chimeras raise thorny ethical questions that the regulatory frameworks currently governing animal research are ill-equipped to answer (especially questions concerning the welfare and wellbeing of modified socially and cognitively complex animals, such as pigs or macaques), and (ii) the justification typically offered for this research – that such research is "necessary" for advancing knowledge of human diseases – is insufficient in these cases. My objective is to raise awareness about the potential harms associated with the creation of such chimeric beings as well as to challenge the adequacy of some current ethical and legal frameworks that regulate animal research.

I-5-652

Creating transgenic pigs to grow organs: A high tech solution to a low tech problem?

L. Syd Johnson
Humanities, Michigan Technological University, Houghton, MI, United States
lsjohnso@mtu.edu

The use of CRISPR to remove PERV DNA from pig cells, and research to create transgenic pigs, have led to speculation about porcine-to-human xenotransplantation. The potential suffering and killing of sensitive, intelligent animals for human benefit requires ethical justification. It's not obvious that using pigs for xenografts would pass ethical muster, and expanding research to develop new ways of using animals warrants careful consideration.

Many patients die while waiting for an organ transplant because too few people choose to donate their organs after death. That problem can be solved by low tech, social engineering rather than the genetic engineering of pigs. Ethical concerns about animal and human welfare in xenotransplantation research are heightened because the organ shortage is a problem that can be solved without it. While the research to grow human-compatible organs in pigs is in its infancy, it is time to consider its ethical implications.

I-5-817

Biotechnology and animal welfare

Bernard Rollin
Colorado State University, Fort Collins, CO, United States
bernard.rollin@colostate.edu

In one of the great ironies of human history, husbandry made domestication of animals possible, which in turn created the possibility of stable civilization, including the development of science and technology. Yet as soon as the technology of the Industrial Revolution was developed, thousands of years of husbandry as ideal and practice were overthrown, and the ancient contract with animals was broken with the development of high technological agriculture that was inimical to fundamental aspects of animal welfare.

If this is true in agriculture, it is *a fortiori* true of animal use in science, where there is *no tradition of animal welfare*, and even animal welfare considerations such as control of pain and distress, presuppositional to good science, were ignored historically. Uncontrolled pain can deform numerous physiological, metabolic, and psychological variables.

This is equally true of biotechnology. As I have demonstrated elsewhere, the advent of biotechnology in agriculture had nothing to do with animal welfare; rather with increasing productivity and profit. We have no reason to believe that biotechnology in science would be any different. Therefore, I would argue, that there needs to be significant regulation of such use to avoid heedlessness to animal suffering.

Theme I: Ethics

Poster Presentations

I-80

Beyond program review: Developing and implementing a quality improvement program for laboratory animal research

John Baumann

Office of Research Compliance, Indiana University, Indianapolis, IN, United States
baumannj@iu.edu

A high functioning office not only maintains it operations with integrity and efficiency, it also undergoes continuous quality improvement. More than the required program review, this presentation will offer a discussion of how an IACUC office has developed and implemented programs of quality improvement in its animal research program including both facility and IACUC offices – QI to improved compliance and/or efficiency and effectiveness. Specific topics include; selection of areas to focus on quality improvement (QI) efforts, implementation of QI activities and programmatic outcomes of QI efforts. Fundamentally, the presentation will describe and assess experiences with QI activities.

I-144

The harm:benefit of primate neurophysiology research – Should it pass the test?

Jarrod Bailey and Katy Taylor

Cruelty Free International, London, United Kingdom
jarrod.bailey@crueltyfreeinternational.org

Harm:benefit analyses underpin NHP research, and are important for NHP neuroscience due to the high welfare costs involved. We show these HBAs are skewed, due to a combination of: an assumption of the human relevance of NHP experiments in the face of evidence to the contrary; exaggeration of their human benefit; playing down the capacity of non-animal methods; overlooking confounding issues like species differences, stress and anaesthesia. Further: claims of essential NHP contributions to medical progress, such as fMRI, deep brain stimulation, and others, are, at best, controversial. We conclude that the ever-increasing power of other neuroscience approaches, such as advances in fMRI and invasive techniques such as electrocorticography and single-unit recordings, renders NHP use redundant. Neuroscience would be more successful for humans if it were conducted with a human focus. We therefore have confidence in opposing NHP neuroscience, both on scientific as well as on ethical grounds.

Reference

Bailey, J. and Taylor, K. (2016). Nonhuman primates in neuroscience research: Scientifically unnecessary. *Altern Lab Anim 44*, 43-69.

I-149

The harm in harm benefit analysis and animal research

David Johnson[1], Louis DeTolla[2], Richard Simmonds[3], Thomas Ferrell[4], Matthew Terzi[2], Jack Dean[5], William Kemp[6], Klaas Kramer[7], Milton April[8], Dennis Johnsen[9], Moshe Bushmitz[10], Paul Houghton[11], Morteza Roodgar[12], Robert Whitney[13] and Lewis Kinter[14,15]

[1]Cascades Biosciences Consultants, Sisters, OR, United States; [2]University of Maryland, School of Medicine, Baltimore, MD, United States; [3]Laboratory Animal Medicine, University of Nevada, Reno, NV, United States; [4]Consultant, Clarks Summit, PA, United States; [5]Drug Development Advisors, LLC, Oro Valley, AZ, United States; [6]Consultant, Sisters, OR, United States; [7]Kramer 3Rs Consultancy, Haarlem, Netherlands; [8]Consultant, Palmer, PA, United States; [9]Consultant, Walnut Creek, CA, United States; [10]Bioculture, Immokalee, FL, United States; [11]Primate Products Inc, Immokalee, FL, United States; [12]Stanford University, Palo Alto, CA, United States; [13]Retired Surgeon General of the United States, Steilacoom, WA, United States; [14]GLP Scientific Consulting, LLC, Unionville, PA, United States; [15]Pharmacolgy & Toxicology, Michigan State University, East Lansing, MI, United States
dkjvet@icloud.com

Exploring the current concepts of Risk Benefit Analysis (RBA) and Harm Benefit Analysis (HBA) is an important ethical issue. RBA is well established in animal research for prospective mitigation of potential adverse effects. HBA was recently introduced as surrogate terminology in the EU and is entering IACUC parlance. RBA is objective, unambiguous, & quantifiable through clinical observation, experimental measurement, and probability analyses. Harm is a subjective, ambiguous, and emotionally loaded term about which well-meaning individuals may reasonably disagree. We posit HBA: 1) Contributes no advantage or improvement over current RBA or animals, investigators, or the public; 2) Miscommunicates by implying that animals are routinely harmed in research, thereby damaging the research community's reputation and recruitment of young scientists; 3) Increases time and cost to gain ethical approvals, delaying critical research and delivery of new medications to patients. Words matter!

I-176

Animal Ethics Committees in Brazil: A survey on the 3Rs concept

Thales de Astrogildo Tréz[1], Rita Leal Paixão[2], Rita de Cassia Maria Garcia[3] and Bianca Marigliani[4]

[1]Instituto de Ciência e Tecnologia, Universidade Federal de Alfenas, Poços de Caldas, Brazil; [2]Departamento de Fisiologia e Farmacologia, Universidade Federal Fluminense, Niterói-RJ, Brazil; [3]Departamento de Medicina Veterinária, Universidade Federal do Paraná, São Paulo, Brazil; [4]Universidade Federal de São Paulo, Brazil

thalestrez@gmail.com

Local Animal Ethics Committees (AECs) in Brazil are responsible, among other things, for evaluating research and teaching protocols. They were established as mandatory collegiates by the Law 11.794/2008, which regulates animal use for scientific purposes. This research applied an online questionnaire, with controlled entrances, to 209 members of AECs from Brazilian federal universities. The results show that knowledge about the 3Rs concept and the Law 11.794 are well spread among those members (especially members with more than two years in AECs). However, the criteria for proper evaluation regarding the number of animals and acceptable practices of euthanasia are not well known; and knowledge about the Go3R platform is extremely low. The research also shows that members recognize the effectiveness of the AECs in promoting the 3Rs concept, but there is a strong need to increase AECs members' knowledge on important aspects of reduction, refinement and replacement of animal use.

References

Bachinski, R., Tréz, T. de A. and Alves, G. G. (2015). Humane education in Brazil: Organisation, challenges and opportunities. *Altern Lab Anim 43*, 337-344.

Bocca, A. L. and Sebben, A. (2009). Uso de animais em pesquisa: O fim justifica os meios. *Brasília Médica 46, Suppl. 1*.

Fiorillo, C. A. and Ferreira, R. M. (2016). Criação e utilização de animais em atividades de ensino e pesquisa científica em face do direito ambiental brasileiro: a lei n. 11.794/2008 e seu enquadramento constitucional. *Revista do Programa de Pós-Graduação em Direito da UFC 36*, 101-115.

Langley, G., Austin, C. P., Balapure, A. K. et al. (2015). Lessons from toxicology: Developing a 21st-century paradigm for medical research. *Environ Health Perspect 123*, 268-272.

Machado, C. J. S., Filipecki, A. T. P., Teixeira, M. O. and Klein, H. E. (2010). A regulação do uso de animais no Brasil do século XX e o processo de formação do atual regime aplicado à pesquisa biomédica. *História, Ciência e Sociedade 17*, 87-105

Tréz, T. de A. (2010). Refining animal experiments: The first Brazilian regulation on animal experimentation. *Altern Lab Anim 38*, 239-244.

Tréz, T. de A. (2014). Knowledge about the 3Rs concept among professors and post-graduate students in Brazil: A survey study. *Altern Lab Anim 42*, 129-136.

Tréz, T. de A. (2015). A caracterização do uso de animais no ensino a partir da percepção de estudantes de ciências biológicas e da saúde. *História, Ciências, Saúde-Manguinhos 22*, 863-880.

Watanabe, M., Fonseca, C. D. and Vattimo, M de F. F. (2014). Instrumental and ethical aspects of experimental research with animal models. *Revista da Escola de Enfermagem da USP 48*, 177-183.

I-223

Muscular dystrophy (MD) studies on dogs: Time for a change?

Emily Trunnell[1] and Alka Chandna[2]

[1]Laboratory Investigations Department, People for the Ethical Treatment of Animals, Norfolk, United States; [2]Laboratory Investigations Department, People for the Ethical Treatment of Animals, Washington, DC, United States

EmilyT@peta.org

In 1981, University of Georgia experimenters observed that two littermates – Rusty and Dusty – exhibited signs of Golden Retriever muscular dystrophy. This observation initiated decades of deliberate breeding of dogs to be afflicted with this disease marked by the progressive degeneration of skeletal and cardiac muscle. As MD ravages their bodies, dogs experience difficulty walking, eating, swallowing, and breathing. Potential treatments in dogs are assessed using measurements of muscle strength and joint contractures. Clinical milestones such as loss of ambulation and the need for ventilator support are tracked. However, researchers acknowledge that after decades of testing on generations of debilitated dogs, there is still no cure or treatment to reverse the course of MD in humans. We consider the global proliferation of colonies of MD dogs, challenges encountered in using dogs, and promising alternative research methods.

References

Kornegay, J. N. et al. (2012). Canine models of Duchenne muscular dystrophy and their use in therapeutic strategies. *Mamm Genome 23*, 85-108.

Kornegay, J. N. (2014). One man's view of one health: Translational lessons learned from a canine model of Duchenne muscular dystrophy. AAVMC 2014 Recognition Lecture, Association of American Veterinary Medical Colleges, 15 March 2014, Alexandria, VA.

Kornegay, J. N. et al. (2014). Pharmacologic management of Duchenne muscular dystrophy: Target identification and preclinical trials. *ILAR J 55*, 119-149.

Serena, E. et al. (2016). Skeletal muscle differentiation on a chip shows human donor mesoangioblasts' efficiency in restoring dystrophin in a Duchenne muscular dystrophy model. *Stem Cells Transl Med 5*, 1676-1683.

Shoji, E. et al. (2015). Early pathogenesis of Duchenne muscular dystrophy modelled in patient-derived human induced pluripotent stem cells. *Sci Rep 5*, 12831.

Young, C. S. et al. (2016). A single CRISPR-Cas9 deletion strategy that targets the majority of DMD patients restores dystrophin function in hiPSC-derived muscle cells. *Cell Stem Cell 18*, 533-540.

I-238

Ethical and scientific arguments against the use of dogs as a second species in toxicity testing

Jarrod Bailey[1], Shiranee Pereira[2], Michael Balls[3] and Michelle Thew[1]

[1]Cruelty Free International, London, United Kingdom; [2]Cruelty Free International, Chennai, India; [3]University of Nottingham, Nottingham, United Kingdom

jarrod.bailey@crueltyfreeinternational.org

Dogs remain the main non-rodent species in preclinical drug tests. This is controversial scientifically, and ethically. Here we argue that there is growing evidence of the lack of human relevance of dog safety tests (the probability of a new drug not being toxic in humans is increased from, say, 70% to just 72% by a negative test result in dogs). Furthermore, there is growing evidence from fMRI data that dogs are more intelligent, socially aware and sentient than previously appreciated. For instance, dogs experience pleasure and positive emotions, empathic-like responses and demonstrate human bonding which, some claim, may be at least comparable to human children. Dogs also respond to emotional cues/valence in human voices, and are able to process faces and facial expressions similarly to humans and monkeys. We therefore contend that using dogs in harmful research, including toxicity testing, is not ethically or scientifically justifiable.

Reference

Bailey, J. and Pereira, S. (submitted). New Scientific evidence indicates that dogs have a level of sentience and cognition comparable to a human child. Can the use of dogs in harmful experiments therefore be justified as ethical?

I-529

A review of IACUC practices at major public U.S. research universities

Ryan Merkley and John Pippin

Research and Regulatory Affairs, Physicians Committee for Responsible Medicine, Washington, DC, United States

rmerkley@pcrm.org

The U.S. Animal Welfare Act (AWA) requires a research facility have an Institutional Animal Care and Use Committee (IACUC), which is charged with AWA compliance for all animal use protocols (U.S. Code § 2143). Prescribed IACUC powers include outright protocol approval, approval with modifications, withholding approval, and suspension of a protocol (CFR 2.31(c)) but we suspect that not all powers are objectively applied. In addition, AWA regulations give IACUCs the power to use designated member review (DMR), in which a single IACUC member grants approval (CFR 2.31(d)). It is unknown how frequently IACUCs employ DMR, but we believe DMR disarms critical functions of IACUC protocol review. Using freedom of information laws, we have requested records from IACUCs of 14 public universities receiving the greatest amount of NIH funding (NIH, 2015). We aim to 1) compile frequencies for the use of specific IACUC protocol powers and the frequency of DMR, and 2) reveal flaws in oversight – in order to improve legal protections for laboratory animals.

References

CFR 2.31 – U.S. Code of Federal Regulations, Title 9, Chapter I, Subchapter A, Part 2, Subpart C, Section 2.31.

NIH – National Institutes of Health (2015). NIH Awards by Location & Organization. Fiscal Year: 2015. https://report.nih.gov/award/index.cfm?ot=&fy=2015&state=&ic=&fm=&orgid=&distr=&rfa=&om=n&pid

U.S. Code § 2143 – U.S. Code, Title 7, Chapter 54, Section 2143(b) – Research facility committee; establishment, membership, functions, etc.

I-559

Evidence-Based Research Network (EBRNetwork) – A call to action for evidence-based research

Marlies Leenaars[1], Karen Robinson[2], Klara Brunnhuber[3], Robin Christensen[4], Maureen Dobbins[5], Bertil Dorch[6], Peg Ford[7], Hans Lund[8], Malcolm Macleod[9], Mona Nasser[10], Hanna Nykvist[11] and Matt Westmore[12]

[1]SYRCLE Radboudumc, Nijmegen, The Netherlands; [2]Johns Hopkins University School of Medicine, Baltimore, United States; [3]BMJ Publishing Group, London, United Kingdom; [4]Odense University Hospital, Odense, Denmark; [5]McMaster University, Hamilton, ON, Canada; [6]University of Southern Denmark, Odense, Denmark; [7]Ovarian Cancer Alliance of San Diego, San Diego, CA, United States; [8]Western Norway University of Applied Sciences, Bergen, Norway; [9]University of Edinburgh, Edinburgh, United Kingdom; [10]Plymouth University, Plymouth, United Kingdom; [11]Bergen University College, Bergen, Norway; [12]University of Southampton, Southampton, United Kingdom

marlies.leenaars@radboudumc.nl

On behalf of the steering committee: Efficient use of earlier research provides a powerful rationale for starting a study and a context in which to set the study results. Explicit use of earlier research, through the conduct of a systematic review, is also necessary for the design of an efficient and informative study. Yet research shows that there is inadequate and biased consideration of earlier research. The EBR Network was initiated in Bergen Norway in December 2014 to promote efficient and explicit use of existing research when new research is planned. The aims, structure and activities of the EBR Network will be presented. Current activities include using peer-reviewed publications and social media to better inform researchers, funders, editors and the public. The "Bergen Statement on Evidence-Based Research" was published in BMJ. The EBR Network is an international collaboration that aims to ensure that no new studies are approved, funded and published without systematic review of the existing evidence; and works towards more efficient production, updating and dissemination of systematic reviews. The EBR Network issues a call for participation.

Reference

Lund, H., Brunnhuber, K., Juhl, C. et al. (2016). Towards evidence based research. *BMJ 355*, i5440. doi:10.1136/bmj.i5440

I-567

Are the 3Rs applied in regulatory toxicity studies?

Katy Taylor and Laura Rego

Science and Regulatory Affairs, Cruelty Free International, London, United Kingdom
katy.taylor@crueltyfreeinternational.org

Regulatory toxicity studies tend to follow standard protocols, however, the extent to which they can still vary with respect to the 3Rs is not known. We recently reviewed a random sample of 300 OECD-compliant studies from the REACH database.

Overall, 49% of studies reported signs or deaths that were consistent with gavage injuries and "gavage-related reflux". 6% of studies reported at least one death from blood sampling. We also found that 10% of studies were performed on corrosive substances, with 22% of these dosing the animals up to the limit dose. 6% of studies overall were performed above the limit dose. In terms of reduction, we found that over 25% of studies used more animals than the minimum.

There are refinements to gavage and blood sampling techniques that could reduce the risk of deaths. There was no association with the date of the studies suggesting that there is still a need for promotion of refinements to toxicity studies to avoid additional suffering.

I-608

Companion animal clinical trials are a potential alternative to laboratory animal experiments but they require equally robust harm-benefit analysis and ethical oversight

Huw Golledge

Universities Federation for Animal Welfare, Wheathampstead, United Kingdom
golledge@ufaw.org.uk

A recent *Nature* editorial argued for the scientific benefits of performing clinical trials on companion animals and suggested they risk being stifled by "overzealous regulations" normally used for laboratory experiments on animals.

I argue that robust regulation and Harm-Benefit Analysis (HBA) of Companion Animal Clinical Trials (CACTs) is even more crucial than for laboratory studies. CACTs entail the possibility of significant harms to participants including side effects of test interventions or their failure to perform as well as standard treatments. These potential harms must be weighed against likely benefit. Furthermore, CACTs involve additional stakeholders (owners and treating clinicians) with unique conflicts of interests between the conduct of the trial and the welfare/longevity of the subjects which should be considered before allowing a CACT. Finally, it is far more difficult to set an endpoint for a companion animal.

I suggest a modified version of the current process of ethical review including HBA, licensing and subsequent monitoring of laboratory animal experiments is the appropriate way to minimise harm and maximise benefit from CACTs.

Reference

Anon. (2016). Pet projects need a helping hand. *Nature 540*, 169.

I-665

Some animals are more equal than others: Implications of the exclusion of mice from the U.S. Animal Welfare Act

Frances Cheng[1] and Alka Chandna[2]

[1]Laboratory Investigations Department, People for the Ethical Treatment of Animals, Norfolk, VA, United States; [2]People for the Ethical Treatment of Animals, Washington, DC, United States
FrancesC@peta.org

In the U.S. and globally, the number of mice used in laboratories has been exploding. However, mice of the genus Mus are excluded from the U.S. Animal Welfare Act (AWA) – the single federal law with legally enforceable regulations stipulating standards of care. Does this exclusion impact mouse welfare? U.S. law mandates that institutions that receive federal funds must comply with federal policies, principles, and guidelines in their treatment of all vertebrate species used in experimentation, testing, and training – including AWA-exempted species. These institutions must report any deviations from the guidelines to the National Institutes of Health (NIH). Using information included in noncompliance reports submitted to the government by the top 25 institutional recipients of NIH research funds, this study analyzes the most common violations of federal guidelines involving mice.

References

Carbone, L. (2004). *What Animals Want: Expertise and Advocacy in Laboratory Animal Welfare Policy*. New York: Oxford University Press.

Ormandy, E. H., Schuppli, C. A. and Weary, D. M. (2009). Worldwide trends in the use of animals in research: The contribution of genetically-modified animal models. *Altern Lab Anim 37*, 63-68.

Taylor, K., Gordon, N., Langley, G. et al. (2008). Estimates for worldwide laboratory animal use in 2005. *Altern Lab Anim 36*, 327-342.

I-700

Maintaining the status quo: U.S. National Institutes of Health squanders opportunity to strengthen protections for nonhuman primates in laboratories

Alka Chandna

Laboratory Investigations Department, People for the Ethical Treatment of Animals, Washington, DC, United States
alkac@peta.org

In 2014, PETA launched a campaign to end psychology experiments at NIH in which newborn monkeys were permanently removed from their mothers and caged by themselves for all but two hours each weekday. The experiments' "Column C" categorization suggested that the animals would experience no pain or distress – even though it is well documented that maternally deprived monkeys suffer from severe and persistent cognitive, social, and physiological deficits. A comprehensive expert review concluded that the experiments had not contributed to treatments for human mental illness even as human-relevant research methods were available. In 2015, NIH pulled the plug on the controversial experiments. Congress, alarmed by the apparent failure of the oversight system, instructed NIH to review the policies and processes governing primate experiments. In spite of Congressional edict and a flood of public comments expressing concern regarding primate use, the workshop fell short – failing to meaningfully include the perspectives of primatologists, bioethicists, or experts in non-animal research methods; and failing to showcase ethical diversity in perspectives.

References

Barr, C. S., Newman, T. K., Shannon, C. et al. (2004). Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biol Psychiatry 55*, 733-738.

Champoux, M., Bennett, A., Shannon, C. et al. (2002). Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Mol Psychiatry 7*, 1058-1063.

Erickson, K., Gabry, K. E., Schulkin, J. et al. (2005). Social withdrawal behaviors in nonhuman primates and changes in neuroendocrine and monoamine concentrations during a separation paradigm. *Dev Psychobiol 46*, 331-339.

Spinelli, S., Schwandt, M. L., Lindell, S. G. et al. (2007). Association between the recombinant human serotonin transporter linked promoter region polymorphism and behavior in rhesus macaques during a separation paradigm. *Dev Psychopathol 19*, 977-987.

Zhang, B., Suarez-Jimenez, B., Hathaway, A. et al. (2012). Developmental changes of rhesus monkeys in response to separation from the mother. *Dev Psychopathol 54*, 798-807.

Theme II – Lessons Learned

Coordinators
Kevin Crofton, US EPA – NCCT, Research Triangle Park, NC, United States
Nathalie Alepée, L'Oréal Research & Innovation, Aulnay sous Bois, France

Oral Presentations

Session II-1: Tox21 10 Year Anniversary

Co-Chairs
Maurice Whelan, Joint Research Center, European Commission, Ispra, Italy
Paul Carmichael, Unilever, Sharnbrook, United Kingdom

II-1-641

A new Tox21 strategic plan and the integration of EPA science

Russell Thomas

National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC, United Statesthomas.russell@epa.gov

The predominant focus of Tox21 collaboration has been on developing and applying high-throughput *in vitro* screening to hazard identification and dose-response. To remain relevant, the interagency collaboration must broaden its focus to developing new predictive toxicology testing approaches for the 21st century. The first part of the talk will outline the new Tox21 strategic plan and common goals of the collaboration. The second part of the talk will focus on evolution of the EPA research program and its integration with the Tox21 collaboration. For EPA, the expanded research activities include significant investments in cheminformatics and computational chemistry, a multi-pronged approach to hazard characterization and prediction, continued improvements in toxicokinetic and exposure modeling, and increased focus on characterizing uncertainty and variability.

This abstract does not necessarily reflect U.S. EPA policy.

II-1-726

Evolution of Tox21 high-throughput screening: Accomplishments and future directions

Menghang Xia

NCATS, NIH, Bethesda, MD, United States
mxia@mail.nih.gov

The Tox21 partnership aims to identify patterns of compound-induced biological response to characterize toxicity/disease pathways, prioritize chemicals for further evaluation, guide optimization of new compounds, and develop predictive models for response in humans. Major accomplishments include the deployment of a comprehensive robotic platform for rapid testing of chemicals in conjunction with the largest collection of environmental chemicals and drugs assembled to date, along with data analysis pipeline and multiple quality-control measures. Deposition into the public domain of the largest-ever dataset has enabled model-building through internal efforts and crowdsourcing. An overview of the Tox21 screening operations setup and accomplishments will be provided, followed by an update on recent efforts to develop models of enhanced biological relevance through stem cell and tissue bioprinting technology development.

II-1-790

The future of Tox21: Changing the NTP landscape

Richard S. Paules

DNTP, NIEHS, NIH, RTP, NC, United States
paules@niehs.nih.gov

Significant advances in toxicology have been achieved through the initial efforts of the first 10 years of the U.S. Tox21 Federal Collaboration. The NTP is striving to incorporate Tox21 approaches into our toxicological characterizations of chemicals to better define the potential adverse effects of exposures on human health. The NTP is engaged in various Tox21 joint collaborations in the new plan for moving Tox21 forward and in NTP-specific Tox21 projects that include the following: 1) using the S1500+ gene set in high-throughput quantitative transcriptomic screens of human liver organoid models to evaluate the role of physiologically-relevant xenobiotic metabolism on responses to chemicals, 2) evaluating *in vitro* models designed to capture population variance in responses to chemical exposures due to genetic differences, 3) evaluation of embryonic zebrafish models in understanding adverse effects of chemicals, and 4) developing and incorporating *in vitro* to *in vivo* extrapolation (IVIVE) approaches to build better linkage between *in vitro* findings and actual risks to human health from chemical exposures.

Session II-2: Government Driven Legislation – EDSP/TSCA

Co-Chairs
Patience Browne, OECD, Paris, France
Sue Marty, The Dow Chemical Company, Midland, MI, United States

II-2-446

Use of alternative methods in the Endocrine Disruptor Screening Program

Patience Browne

Test Guidelines Programme, OECD, Paris, France
patience.browne@oecd.org

The US EPA's Endocrine Disruptor Screening Program (EDSP) has evaluated *in vitro* high throughput screening (HTS) assays for estrogen (ER) and androgen receptor(AR) targets. The multiple HTS ER and AR assays available through EPA's ToxCast Program employ a variety of *in vitro* technologies and contribute to the confidence of the predicted endocrine activities. The performance of the HTS assays against large sets of reference chemicals has been sufficient to use resulting data in hazard identifications, and these performance analyses have elucidated potential limitations of assays traditionally considered "gold standards". Endocrine targets addressed by single assays may not have the same associated scientific confidence as a suite of assays measuring the same endpoint; however, *in vitro* data provide mechanistic insight and contribute to the understanding of the chemical's mode of action in an AOP context. The recent EPA acceptance of alternative data has generated momentum for finding non-animal approaches, used alone or in combination, for *in vivo* endpoints and to develop more sophisticated predictive models.

II-2-445

The effects of active transport in an *in silico-in vitro* based risk assessment approach for potential endocrine disrupting substances

Barbara Birk, Caroline Gomes, Eric Fabian, Rene Zbranek, Christian Haase, Tzutzuy Ramirez Hernandez, Bennard van Ravenzwaay and Robert Landsiedel

Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany
barbara.birk@basf.com

In order to use results of non-animal testing for a risk assessment, effect concentrations obtained from *in vitro* assays need to be translated to external exposure. *In silico* physiologically-based toxicokinetic (PBTK) models can be a tool for this *in vitro* to *in vivo* extrapolation (IVIVE). We used IVIVE to calculate oral lowest observed effect levels (LOEL) based on effect concentration in *in vitro* tests for 10 substances for endocrine endpoints: Compared to published *in vivo* derived LOELs, *in vitro*-based predictions of LOELs were in the same order of magnitude for 6 of the 10 substances. 3 Substances which's LOELs were correctly predicted or over/under predicted were investigated for potential active transport by efflux transporters (MDR1-Pgp, BCPR) and three organic cation/anion uptake transporters (OATP1B1, OAT1, OCT2). Since distribution is driven by diffusion in routine PBTK-models, it is assessed here if active transport may be responsible for failed IVIVEs. The impact of these data on of the outcome of the IVIVE calculation was assessed.

II-2-627

How do laws and policies regulating chemicals drive alternative methods development?

Kristie Sullivan

Physicians Committee for Responsible Medicine, Washington, DC, United States

ksullivan@pcrm.org

Legislation influences policies related to the use of animals in laboratories, traditionally through regulations governing the care and welfare of animals via laws such as the U.S. Animal Welfare Act. However, legislation intended to regulate the commercial use of chemicals or other products can also have a profound effect on animal use in laboratories, by directly or indirectly encouraging or requiring the use of alternatives to *in vivo* toxicological tests. This presentation will review U.S. laws, such as the Toxic Substances Control Act/Lautenberg Chemical Safety Act, and programs developed through Congressional mandate, such as the Endocrine Disruptor Screening Program, and examine their current and potential impacts on the development and use of *in vitro* and computational methods. Where relevant, they will be compared to International and EU laws and projects with similar policy goals, such as REACH and the OECD Test Guidelines Programme.

II-2-527

Implications of the recent 2016 amendment of the Toxic Substances Control Act (TSCA) on the development and implementation of non-animal methods

Catherine Willett

The Humane Society of the United States, Gaithersburg, MD, United States

kwillett@humanesociety.org

The Frank R. Lautenberg Chemical Safety for the 21st Century Act (LCSA) increases the authority of the Environmental Protection Agency (EPA) to obtain information on new and existing industrial chemicals and could result in vast increases in animal testing. The amendment also requires both EPA and "any person" developing information under the Act to reduce and replace vertebrate testing and requires EPA to "promote the development and timely incorporation" of non-animal methods. Existing chemicals are subject to prioritization and "high priority" chemicals must undergo risk evaluation, both under tight deadlines. The combined pressures of tight deadlines and reducing animal use offers an opportunity for increased implementation of non-vertebrate evaluation tools and requires coordinated efforts between industry, agency scientists and regulators, and other stakeholders to leverage existing approaches from other sectors and expand available methods.

II-2-730

Cues from EDSP: Earlier screening for endocrine activity

Sue Marty

Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, United States

mmarty@dow.com

Detection of endocrine disrupting activity in both agricultural and industrial chemicals is a goal of global regulatory programs. The US EPA's Endocrine Disruptor Screening Program (EDSP) is focused on potential effects on estrogen, androgen, thyroid and steroidogenesis pathways. Companies are establishing their own internal programs to pre-screen compounds for endocrine activity. Dow is developing an integrated, multi-tiered approach that includes cheminformatics (*in silico* models, including QSARs), bioprofiling (alternative models, including *in vitro*), *in silico* toxicokinetics and, when needed, *in vivo* screening to evaluate potential endocrine activity. Earlier screening will result in less animal use, both through avoidance of *in vivo* endocrine screening/testing, as well as through the use of targeted *in vivo* testing when needed.

Session II-3: Government Driven Legislation – Cosmetics

Co-Chairs
Vanessa Rocha, Natura, Cajamar, Brazil
Rob Taalman, Cosmetics Europe, Brussels, Belgium

II-3-236

Safety assessment for cosmetics

Rob Taalman[1], Nathalie Alépée[2], Takao Ashikaga[3], Elodie Clouet[4], Magalie Cluzel[5], Aurelia Del Bufalo[2], Carsten Goebel[6], Sebastian Hoffmann[7], Petra Kern[8], Martina Klaric[1], Jochen Kuehnl[9], Karsten R. Mewes[10], Masaaki Miyazawa[11], Dirk Petersohn[10], Erwin Van Vliet[12], Nicola Gilmoure[13], Jalila Hibatallah[14] and Lauren Hutchison[15]

[1]Cosmetics Europe, Brussels, Belgium; [2]L'Oréal Research & Innovation, Aulnay sous Bois, France; [3]Shiseido, Kanagawa, Japan; [4]Pierre Fabre, Castres, France; [5]LVMH, St Jean de Braye, France; [6]Coty, Darmstadt, Germany; [7]seh consulting+services, Paderborn, Germany; [8]Procter & Gamble, Strombeek-Bever, Belgium; [9]Beiersdorf AG, Hamburg, Germany; [10]Henkel AG, Düsseldorf, Germany; [11]KAO, Tochigi, Japan; [12]SeCAM, Magliaso, Switzerland; [13]Unilever, Bedford, United Kingdom; [14]Chanel Parfums Beauté, Neuilly sur Seine, France; [15]Colgate-Palmolive, Piscataway, NJ, United States

rtaalman@cosmeticseurope.eu

The Cosmetic Europe's skin tolerance task force aims to develop regulatory accepted test strategies that enable cosmetic industries to conduct skin sensitisation safety assessments without the use of animals. For this purpose, we conducted a case study to explore how defined approaches might be combined with *in silico* models, bioavailability data and exposure considerations to support meaningful safety assessments of topical ingredients. Risk assessment situations for ingredients with different skin sensitizing potency and exposure scenarios will be discussed to explore what type of test data and information is required to perform animal-free risk assessment for regulatory purposes. In addition, the identified difficulties and questions arising will be discussed such as; uncertainty considerations, data discrepancies and the acceptance by regulators of new approach methodologies.

II-3-620

Alternative methods for risk assessment: Brazil takes off

Vanessa Rocha

Product Safety, Natura Cosmetics, Cajamar, Brazil
vanessarocha@natura.net

Brazil has been a leader country on Latin America to implement alternative methods to animal testing. Since 2008 it has been a growing concern about using animals for product development and regulatory testing, especially for cosmetics. The drive for this change resulted first in the implementation of CONCEA Resolutions n.18 (2014) and n.31 (2016) which should reduce the need of using animals in tests required for the registration of drugs, agrochemicals, cleaning products and cosmetics, on outcomes as skin/ocular irritation, skin sensitization, genotoxicity, among others. Discussions on Deputies bill 70/2014 also intend to ban the sale of cosmetic products and ingredients newly tested on animals anywhere in the world after 3 years. In this scenario, professionals from companies, academia, and government should use alternative methods for hazard assessment. In this presentation, a case study on risk assessment of essential oils for cosmetics, using *in silico* and *in vitro* approaches will be discussed, illustrating some of the new challenges and possible ways to overcome limitations.

References

Api, A. M., Belsito, D., Bruze, M. et al. (2015). Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. *Food Chem Toxicol 82, Suppl*, S1-S19.

CONCEA – Conselho Nacional de Controle de Experimentação Animal (2014). Resolução Normativa CONCEA nº 17, de 3 de Julho de 2014. Dispõe sobre o reconhecimento de métodos alternativos ao uso de animais em atividades de pesquisa no Brasil e dá outras providências.

CONCEA (2014). Resolução Normativa CONCEA nº 18, de 24 de Setembro de 2014. Reconhece métodos alternativos ao uso de animais em atividades de pesquisa no Brasil, nos termos da Resolução Normativa nº 17, de 3 de Julho de 2014, e dá outras providências.

II-3-431

Using high-throughput literature mining to support read-across predictions of skin sensitization

Grace Patlewicz[1], Nancy Baker[2], Thomas Knudsen[1] and Kevin Crofton[1]

[1]National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park (RTP), NC, United States; [2]Leidos, Research Triangle Park, NC, United States

patlewicz.grace@epa.gov

Read-across predictions require high quality measured data for source analogues. These data are typically retrieved from structured databases, but biomedical literature data are often untapped because current literature mining approaches resource intensive. Our high-throughput (HT) literature mining methods use MeSH terms to convert unstructured literature to a structured format. Using these HT methods, we built a literature profile for skin sensitization. We selected a target chemical (2E-decenal) and searched for source analogues based on reaction chemistry. Literature data for the source analogues were visualized as LitToxPIs to read-across the sensitization potential of 2E-decenal. Applicable across endpoints, our HT methods provide data sources to improve scientific confidence in read-across predictions.

The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

II-3-563

Safety assessment of topical ingredients – A case study

Nathalie Alépée[1], Takao Ashikaga[2], Elodie Clouet[3], Magalie Cluzel[4], Aurélia Del Bufalo[1], Nicola Gilmour[5], Carsten Goebel[6], Jalila Hibatallah[7], Sebastian Hoffmann[8], Lauren Hutchison[9], Petra Kern[10], Jochen Kuehnl[11], Karsten R. Mewes[12], Masaaki Miyazawa[13], Erwin Van Vliet[14], Martina Klaric[15] and Dirk Petersohn[16]

[1]L'Oréal Research & Innovation, Aulnay sous Bois, France; [2]Shiseido, Kanagawa, Japan; [3]Pierre Fabre, Castres, France; [4]LVMH, St Jean de Braye, France; [5]Unilever, Bedford, United Kingdom; [6]Coty, Darmstadt, Germany; [7]Chanel Parfums Beauté, Neuilly sur Seine, France; [8]seh consulting+services, Paderborn, Germany; [9]Colgate-Palmolive, Piscataway, NJ, United States; [10]Procter & Gamble, Strombeek-Bever, Belgium; [11]Beiersdorf AG, Hamburg, Germany; [12]Henkel AG, Düsseldorf, Germany; [13]Kao Corporation, Tochigi, Japan; [14]SeCAM, Magliaso, Switzerland; [15]Cosmetics Europe, Brussels, Belgium; [16]Henkel AG, Düsseldorf, Germany

nalepee@rd.loreal.com

The Cosmetic Europe's skin tolerance task force aims to develop regulatory accepted test strategies that enable cosmetic industries to conduct skin sensitization safety assessments without the use of animals. For this purpose, we conducted a case study to explore how defined approaches might be combined with *in silico* models, bioavailability data and exposure considerations to support meaningful safety assessments of topical ingredients. Risk assessment situations for ingredients with different skin sensitizing potency and exposure scenarios will be discussed to explore what type of test data and information is required to perform animal-free risk assessment for regulatory purposes. In addition, the identified difficulties and questions arising will be discussed such as uncertainty considerations, data discrepancies and the acceptance by regulators of new approach methodologies.

II-3-543

Comparison of *in vitro* metabolism models for cosmetics relevant prediction of skin absorption and toxicity

Daniela Lange[1], Carine Jaques-Jamin[2], Joan Eilstein[3], Helen Duplan[2], Camille Genies[2], Sébastien Gregoire[3], Richard Cubberley[4], Corie Ellison[8] Martina Klaric[5], Amy Roe[8], Helga Rothe[6], Sabrina Salhi[7], Nicola Hewitt[5] and Andreas Schepky[1]

[1]Beiersdorf AG, Hamburg, Germany; [2]Pierre Fabre Dermo-Cosmétique, Toulouse, France; [3]L'Oréal, Aulnay sous Bois, France; [4]Unilever, Sharnbrook, United Kingdom; [5]Cosmetics Europe, Brussels, Belgium; [6]Coty, Darmstadt, Germany; [7]GSK, Paris, France; [8]The Procter and Gamble Co., Cincinnati, OH, United States

daniela.lange@beiersdorf.com

The Cosmetics Europe ADME Task Force was set up to improve the animal-free measurement and prediction of the bioavailability of topically applied chemicals for safety assessment. Toxicity of these chemicals is driven by the potential of penetration into and through skin, as well as possible metabolism in skin. We used subcellular S9 fractions of reconstructed human skin, as well as *ex vivo* pig and human skin to measure skin metabolism and compare the results to hepatic metabolism measured by hepatic S9 fractions. Subcellular S9 fractions tests serve as an initial screen for potential metabolism, before comprehensive metabolism assays using *ex vivo* viable skin can be carried out. Data has been generated for 50 cosmetic-relevant chemicals for which skin penetration, partitioning experiments and reactivity assays have also been carried out. The combination of these types of data will be used to develop approaches to predict bioavailability and local toxicity following topical application.

II-3-796

Practical integrated testing strategy for skin sensitization assessment

Masaaki Miyazawa

Kao Corporation, Tochigi, Japan

miyazawa.masaaki@kao.co.jp

Integrated testing strategies (ITS) using multiple non-animal test methods have been developed to predict sensitizing potential and potency of chemicals. A negative result in DPRA, KeratinoSens (KS) and h-CLAT methods (3 out of 3 ITS) predicts a chemical to be a non-sensitizer. Our goal is to develop a practical ITS using test method combinations. The binary test battery with KS and h-CLAT, which had the sensitivity similar to that of the 3 out of 3 ITS by excluding predictive limitations, and the addition of DPRA test gave a firm conclusion of "non-sensitizer"; good first tiers of a bottom-up approach. With a positive result observed in any of the 3 tests, the chemical would be deemed a "sensitizer". Even when a sensitizer was predicted, Bayesian Network ITS-3 decisions fell into one category difference in LLNA and the conservative classification from minimal to high potency was assigned. Based on these results, the workflow is designed to support a practical skin sensitization assessment.

Reference

Jaworska J., Natsch, A., Ryan, C. et al. (2015). Bayesian integrated testing strategy (ITS) for skin sensitization potency assessment: A decision support system for quantitative weight of evidence and adaptive testing strategy. *Arch Toxicol 89*, 2355-2383.

II-3-636

"PRISME T/NT": A 2D/3D molecular descriptor-based model for predicting acute systemic toxicity of reactive compounds?

Reine Note[1], Delphine Blanchet[2], Hicham Noçairi[1], Renaud Bouchardon[1], Jérôme Gomar[1], Charles Gomes[1], Jacques Clouzeau[2] and Silvia Teissier[1]

[1]L'Oréal Research & Innovation, Paris, France; [2]L'Oréal, Safety Evaluation-Research & Innovation, Paris, France

rnote@rd.loreal.com

The primary driver for conducting acute toxicity studies responds to classification and labeling purposes. Many efforts have been gathered in order to develop alternatives for the safety assessment of innovative ingredients or propose a testing strategy (ECVAM 2014) that would comply with the 3R's principles and animal testing ban. Tangible results of the partnerships that L'Oréal put in place in this direction include the construction of a toolbox containing *in silico* (structural alerts) and *in vitro*-models. However, such models do not fully cover the complexity of ingredients bearing intrinsic chemical reactivity. We thought that relying on 2D/3D molecular descriptors could be a smart way to tackle this issue and enrich our toolbox. A statistical model has been developed and validated with a set of 185 (non)proprietary compounds known to be highly reactive. We will present herein the results obtained and show how such results could be used to build an integrated testing strategy.

Session II-4: Eye Hazard Potential Examples

Co-Chairs
Anne Gourmelon, OECD, Paris, France
Nathalie Alépée, L'Oréal Research & Innovation, Aulnay sous Bois, France

II-4-447

Development of an integrated approach to testing and assessment for eye irritation potential in OECD

Anne Gourmelon
OECD, Paris, France
anne.gourmelon@oecd.org

In 2014, the United States EPA expressed willingness to have a new OECD document serving as a cross-walk across the plethora of new ocular toxicity guidance documents and Test Guidelines. The available and foreseeable *in vitro* and *ex vivo* assays were expected to contribute to an Integrated Approaches to Testing and Assessment (IATA) for classification for serious eye damage/eye irritation endpoints according to the Globally Harmonised System, together with other relevant sources of information. The European Commission joined the endeavour based on their broad experience promoting alternatives in the context of the Cosmetics Directive. Three years later, a Guidance Document is approved, including a decision logic to optimise selection of tests when more data is needed. The document is inclusive of methods that have not been validated so far, but that offer perspectives for improving prediction of eye irritants (GHS Cat. 2), or that could be more easily accessible geographically or economically.

Reference

OECD (2017). Draft Guidance Document on Integrated Approach to Testing and Assessment for Eye Irritation Potential. ENV Publications, Series on Testing and Assessment. http://www.oecd.org/env/ehs/testing/Draft%20IATA_Eye_Hazard_v22Dec2016_cleared_v2.pdf

II-4-565

CON4EI: Development of testing strategies for hazard identification and labelling of eye irritating chemicals

Els Adriaens[1], Sandra Verstraelen[2], Nathalie Alépée[3], Helena Kandarova[4], Przemyslaw Fochtman[5], Robert L. Guest[6], Jamin Willoughby[7] and An Van Rompay[8]

[1]Adriaens Consulting bvba, Aalter, Belgium; [2]Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium; [3]L'Oréal, Research & Innovation, Aulnay sous Bois, France; [4]MatTek, Bratislava, Slovakia; [5]Institute of Industrial Organic Chemistry, Pszczyna, Poland; [6]Envigo, Cambridge, United Kingdom; [7]Cyprotex US, LLC, Kalamazoo, MI, United States; [8]Vito, Antwerp, Belgium
adriaens.consulting@telenet.be

The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation) project was to develop tiered testing strategies for eye irritation assessment that can lead to complete replacement of the *in vivo* Draize rabbit eye test (OECD TG 405). A set of 80 reference chemicals (e.g. balanced by driver of classification and physical state), was tested with seven test methods. Three different strategies that distinguish between Cat 1 and Cat 2 chemicals and chemicals that do not requiring classification (No Cat), were suggested. A standalone test method (EpiOcular ET-50), a two-step and three-step strategy that use an RhCE test method (EpiOcular EIT or SkinEthic™ HCE EIT) at the bottom (identify No Cat) in combination with the BCOP LLBO (two-step strategy) or BCOP OP-KIT and SMI (three-step strategy) at the top (identify Cat 1). Cat 1 sensitivities between 71.1% and 82.9%, Cat 2 sensitivity between 64.2% and 68.5% were obtained with a specificity of at least 80%. Similar results were obtained for the Top-Down and Bottom-Up approach.

II-4-463

Cosmetics Europe analysis of the robustness of testing strategies for UN GHS classification for serious eye damage/eye irritation of chemicals

Nathalie Alépée[1], Els Adriaens[2], Daniel Bagley[3], Bertrand Desprez[4], Jalila Habatallah[5], Karsten R. Mewes[6], Uwe Pfannenbecker[7], Àlvar Sala[8] and Pauline McNamee[9]

[1]L'Oréal, Research & Innovation, Aulnay sous Bois, France; [2]Adriaens Consulting, Aalter, Belgium; [3]Colgate-Palmolive, Piscataway, NJ, United States; [4]Cosmetics Europe, Brussels, Belgium; [5]Chanel Parfums Beauté, Neuilly sur Seine, France; [6]Henkel AG, Düsseldorf, Germany; [7]Beiersdorf AG, Hamburg, Germany; [8]Kao Corporation S.A., Barcelona, Spain; [9]The Procter & Gamble Company, Egham, United Kingdom
nalepee@rd.loreal.com

Cosmetics Europe (CE) eye programme hasa current focus on demonstrating robustness of testing strategies/approaches for identification of serious eye damage/eye irritation of chemicals that can be advocated for external/regulatory acceptance. To enable this, CE curated an initial database of chemicals for which *in vivo* and partial *in vitro* data exist. This database was used for selection of 80 chemicals, based on *in vivo* drivers of classification, tested in *in vitro* test methods in the CEFIC CON4EI project. Working on an industry platform level, such newly generated *in vitro* data were integrated into the initial database. Remaining *in vitro* data gaps were then identified and testing completed by CE to build a comprehensive *in vivo*/*in vitro* database of more than 110 chemicals to date. Building on proposed CON4EI testing strategies, CE has analysed the comprehensive database to determine the robustness of such testing strategies and to identify where opportunities exist for refinement.

II-4-112

Lessons learned: US EPA Office of Pesticide Programs acceptance of alternative eye methods

Amy Clippinger[1], Erin Hill[2], Rodger Curren[2], David Allen[3] and Patricia Bishop[1]

[1]PETA International Science Consortium Ltd, London, United Kingdom; [2]Institute for In Vitro Sciences, Gaithersburg, MD, United States; [3]ILS, Research Triangle Park, NC, United States

AmyJC@piscltd.org.uk

A joint effort between industry, NGOs, and the EPA has led to the development of a decision tree approach using three *in vitro*/*ex vivo* assays to determine eye irritation under the EPA Office of Pesticide Programs' (OPP) hazard classification and labeling system. A policy document issued by the EPA in 2013 and updated in 2015 describes the approach that industry could apply to new registrations of antimicrobial cleaning products (AMCPs) and, on a case-by-case basis, of conventional pesticide products. This testing strategy has been successfully applied to support the registration of new AMCPs; however, it has been significantly underutilized. Barriers, including reviewer variability and lack of international harmonization, were identified. This presentation will discuss recent steps taken to address these barriers and to expand use of the alternative strategy to the evaluation of conventional pesticides. Insights revealed during this project that could improve the implementation of similar alternatives efforts in the future will also be discussed.

II-4-369

Development of the *in vitro* assay for evaluating reversible eye irritation

Naoki Yamamoto[1], Noriko Hiramatsu[1], Yoshinao Kato[2] and Hajime Kojima[3]

[1]Laboratory of Molecularbiology and Histochemistry, Fujita Health University Institute of Joint Research, Toyoake, Japan; [2]General Institute, Nippon Menard Cosmetic Co., Ltd, Nagoya, Japan; [3]Div. Risk Assessment, National Institue of Health Sciences (NIHS), Tokyo, Japan

naokiy@fujita-hu.ac.jp

Differences in reversibility of cornea damages could not be distinguished in previously developed non-animal alternative eye irritation methods. Whether the eye damage caused in reversible or irreversible is an important hazard assessment criterion such as GHS category 2A or 2B.

Based on the performance standard in OECD TG 492, the protocol was developed using a three-dimensional cornea model with cloned iHCE-NY1 cell line, which was derived from corneal epithelial cells isolated from human corneal tissue and transfected with immortalized gene (SV40 Large T antigen). To evaluate reversible cytotoxicity of test substances, the MTT assay and pathological finding were examined with the cornea model on day 1, 7 and 14 of post-culture.

This protocol induced reversible responses at four test chemicals in eight ones classified by GHS category 2A or 2B. These results address the post-incubation of RhCE model after applying test chemicals may be useful to evaluate reversible cornea damage.

II-4-372

Studying innate immunity mechanisms of the human corneal epithelium with SkinEthic™ HCE cmm

Barbara De Servi[1], Christian Pellevoisin[2], Stefano Barabino[3] and Marisa Meloni[3]

[1]Clinica Oculistica, Di.N.O.G.M.I., Azienda Ospedaliera Universitaria San Martino-IST, Genoa, Italy; [2]EPISKIN Academy, Lyon, France; [3]VitroScreen SrL-In Vitro Research Laboratories, Milan, Italy

cpellevoisin@episkin.com

The corneal epithelium, the outermost layer of the cornea, is organized to form an effective barrier against fluid loss and pathogen penetration. The occular surface is potentially exposed to harmful materials such as chemicals, medical devices, cosmetics and also bacteria or virus… The SkinEthic™ HCE model is a reconstructed human corneal epithelium (HCE) without Bowman's layer and stroma. The biological relevance of this model is recognized to study exposure to chemicals, drug penetration or to model some diseases such as dry eye syndrome (Barabino et al., 2017). The new SkinEthic™ HCE cmm model used in this work has been designed to enlarge application of this corneal model to study cells migration associated to immuno-inflammatory responses. The epithelium is reconstructed in a modified insert that allows cells migration through the polycarbonate membrane (Pellevoisin et al., 2016). without impacting the reconstruction of the epithelium.

The SkinEthic™ HCE cmm model is cultivated with activated immunocompetent cells (monocyte cell line -THP-1) to study infiltration of the corneal tissue in response to ocular surface damage and inflammation in modified culture conditions. Undifferentiated THP-1 cells are cultured on the lower face of the transwell filter (polycarbonate membrane) and once an adherent cell layer is formed, the immunocompetent cells infiltration and migration process is studied at different time points of post incubation (4-24-48 h). Cells infiltration is monitored by labelling of immune cells (Immunohistochemistry of CD68, CD83 and CD14) and epithelium responses by histology and gene expression variation (qRT-PCR of several genes: MMP9, TNFα, AQP3, MUC4, ZO1, OCLN, ITGB1, TGFB1, IL8).

The presented ocular surface immunocompetent system demonstrates to be able to monitor macrophages and dendritic cells migration into the corneal tissue underling their differential positioning and immunological role within the epithelium. The model is under development to investigate immunomodulatory therapies to effectively treat some inflammatory based-ocular diseases.

References

Barabino, S., De Servi, B., Aragona, S. et al. (2017). Efficacy of a new ocular surface modulator in restoring epithelial changes in an in vitro model of dry eye syndrome. *Curr Eye Res 42*, 358-363.

Pellevoisin, C., Douillard-Guilloux, G., Sahuc F. and Ancellin N. (2016). The use of Skinethic reconstructed human epidermis (RHE) to study immunocompetent cells trafficking and recruitment between skin compartments. SID congress.

Session II-5: Read Across

Co-Chairs
Grace Patlewicz, US, EPA, RTP, NC, United States
Masamitsu Honma, National Institute of Health Sciences, Tokyo, Japan

II-5-432

Navigating through the minefield of read-across: From research to practical tools

Grace Patlewicz[1], George Helman[2], Prachi Pradeep[2] and Imran Shah[1]

[1]National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, United States;
[2]Oak Ridge Institute for Science Education (ORISE), Oak Ridge, TN, United States
patlewicz.grace@epa.gov

Read-across is used for regulatory purposes as a data gap filling technique. Research efforts have focused on the scientific justification and documentation challenges involved in read-across predictions. Software tools have also been developed to facilitate read-across prediction. We highlight a handful of the publicly available category workflow read-across tools to articulate their respective capabilities. Whilst these tools address a number of the workflow steps, few consider an uncertainty assessment. We will present an algorithmic, automated read-across approach using *in vitro* bioactivity data (from EPA's ToxCast program) and chemical descriptor information to predict *in vivo* toxicity effects. We will demonstrate how read-across predictions can be evaluated to quantify uncertainty. We showcase progress in translating these efforts into practical tools.

The abstract does not reflect EPA policy.

II-5-645

Read-across: Lessons learned & success stories from Canada's Chemicals Management Plan

Sunil Kulkarni and Matthew Gagne
Health Canada, Ottawa, ON, Canada
sunil.kulkarni@hc-sc.gc.ca

Canada's Chemicals Management Plan (CMP) is a world-leading regulatory program that integrates science-based approaches aimed at protecting human health and environment. Under the 2nd and 3rd phases of CMP, a significant portion of substances being assessed for human health risks have little or no conventional toxicological data. Lack of empirical toxicity data on these chemicals posed a significant challenge for carrying out their risk assessment. Examples of how read-across, a non-animal test method, was successfully applied in risk assessments of certain CMP chemicals is discussed. Read-across within CMP program continues to evolve, including integrating available data from toxicogenomics and other emerging data (e.g. ToxCast). Based on experiences and lessons learned an in-house guidance was prepared to streamline application of read-across, capture uncertainties and to ensure that approach is applied consistently in a scientifically defensible manner in CMP health risk assessments.

II-5-561

Our recent experiences for development of read-across approach for chemical safety assessment

Takashi Yamada, Akihiko Hirose and Masamitsu Honma
National Institute of Health Sciences, Tokyo, Japan
t-yamada@nihs.go.jp

Many efforts on developing read-across assessment have been made internationally to promote the regulatory application. Here we will share our recent experiences on development of read-across for chemical safety assessment. First topic is ICCA-LRI and NIHS workshop 2016 in Japan. Panel discussion concluded that the biggest barriers to widespread application of read-across are insufficient documentation of validation, limited number of successful case studies, lack of globally recognized guidelines and limited number of regulators with experience in read-across. Second topic is the OECD IATA case studies project since 2015. The objective is to increase experience with the use of IATA by developing case studies that are fit for regulatory use. Through the review of each case study, two areas with high priority were identified for further guidance development; definition of analogues/category boundaries and uncertainty analysis and reporting. Read-across is conceptually simple but practically needs wide range of expertise. Developing more case studies and sharing the experiences are a promising way for expanding the use of read-across.

II-5-671

Benefits of using read across and *in silico* techniques to fill non-SIDS data gaps for high production volume chemical categories

Francis Kruszewski and Kathleen Stanton
American Cleaning Institute, Washington, DC, United States
fkruszewski@cleaninginstitute.org

The American Cleaning Institute (ACI) has engaged in the voluntary global International Council of Chemical Associations and U.S. Environmental Protection Agency high production volume chemical programs. ACI compiled a baseline set of health and environmental effects data, representing the OECD screening information data set (SIDS), for 260 surfactant chemicals within nine chemical consortia. Due to the structural similarity of the chemicals within a category read across and *in silico* techniques were used to fill data gaps. While not required ACI consortia also compiled non-SIDS data for many of these substances. The non-SIDS data endpoints included skin and eye irritation, dermal sensitization, carcinogenicity, ADME, and chronic aquatic toxicity. The benefits realized in using read across and *in silico* techniques for non-SIDS data were the opportunity to reduce animal testing and related costs, strengthen category concepts, and enhance public access to hazard data for chemicals.

II-5-126

Case study of read-across predictions using a Generalized Read-Across (GenRA) approach

George Helman[1], Imran Shah[2] and Grace Patlewicz[2]

[1]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN, United States; [2]National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park (RTP), NC, United States

helman.george@epa.gov

We developed the Generalized Read-Across (GenRA) approach to facilitate automated, algorithmic read-across predictions. GenRA uses *in vitro* bioactivity data in conjunction with chemical information to predict up to 574 different apical outcomes from repeat-dose toxicity studies. Here, we use a case-study approach to characterize GenRA read-across predictions for a group of reference chemicals. We highlight examples where physicochemical parameters such as LogKow are helpful in refining the read-across predictions. These efforts demonstrate the utility of automated approaches for chemical analogue selection using algorithmic read-across approaches.

EPA disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

II-5-632

Application of read-across in quantitative chemical risk assessment in a regulatory setting

Q. Jay Zhao

Office of Research and Development, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH, United States

zhao.jay@epa.gov

Human health risk assessments are often requested for chemicals found in environmental media at contaminated sites that have limited or no available toxicity information. Consequently, these chemicals do not have quantitative toxicity values and therefore are not considered in the calculation of a hazard or risk metric and do not inform clean-up decisions. To address this issue, we have applied an expert-driven tiered read-across approach for identifying the most appropriate chemical analog and adopting the corresponding quantitative value (e.g., point-of-departure) as a surrogate to inform dose-response assessment of a target chemical. This tiered surrogate approach significantly advanced quantitative risk assessment for data-poor chemicals and filled a critical gap in regulatory remediation activities. Case studies will be presented to demonstrate application of the tiered read-across approach and highlight ongoing challenges.

The views expressed in this abstract are those of the author and may not necessarily reflect the views or policies of the U.S. EPA

Session II-6: Integrating *In Vitro* to Predict Organ Toxicity

Co-Chairs
Frank Sistare, Merck & Co., Inc., West Point, PA, United States
Yaakov Nahmias, Hebrew University, Jerusalem, Israel

II-6-759

Integrating advanced *in vitro* liver models to reduce risks of drug induced liver injury (DILI) in pharmaceutical development

Frank Sistare
SALAR, Merck & Co., Inc., West Point, PA, United States
frank_sistare@merck.com

In vitro liver test systems are becoming increasingly sophisticated, durable, and stable. When integrated together with novel molecular end points, computational models, and translational clinical biomarkers, they can be used to enhance confidence in predicting liver safety. Emerging drug development strategies have generally evolved to inform DILI risk for reactive metabolite formation, mitochondrial toxicity, alteration of bile salt homeostasis, and cellular imaging endpoints. Critical to the adoption of such models is favorable data from a phased testing approach of characterization, evaluation, qualification, situational deployment, and then a staged plan for more routine implementation. Expectations are high for a future state with more predictive tools and problem-solving strategies that will enable earlier and better decisions, reduce costs, prevent wasted animal and human resources, accelerate drug development success, and make it less dependent on animal studies.

II-6-341

Developing the next generation of organ on chip technology

Yaakov Nahmias[1,2]
[1]Hebrew University, Jerusalem, Israel; [2]Tissue Dynamics Ltd, Jerusalem, Israel
ynahmias@gmail.com

Organ-on-chip technology aims to replace animal toxicity testing, but thus far demonstrated few advantages over traditional methods. Current methods to evaluate toxicity rely on end-point assays, resulting in limited kinetic and mechanistic information. We present the Tissue Dynamics platform capable of maintaining vascularized 3D liver tissue for over a month *in vitro*. Tissues acquire physiological structure and show complex metabolic zonation. Tissue-embedded metabolic sensors permit the real-time assessment of cellular function. Change in metabolic function is the first indication of stress, preceding any detectable damage. We show a new CYP450-idependent mechanism of acetaminophen toxicity that may be responsible for clinically observed nephrotoxicity. We also show troglitazone-induced metabolic changes that might underlie its observed idiosyncratic toxicity. Our work marks the importance of tracing function in *real time*, demonstrating specific advantages in predicative toxicology.

References

Bavli, D., Prill, S., Ezra, E. et al. (2016). Real-time monitoring of metabolic function in liver-on-chip microdevices tracks the dynamics of mitochondrial dysfunction. *PNAS 113*, E2231-E2240.

Levy, G., Bomze, D., Heinz, S. et al. (2015). Long-term culture and expansion of primary human hepatocytes. *Nat Biotechnol 33*, 1264-1271.

II-6-111

An integrated testing strategy using weight of evidence approach for identifying novel structural associations with skin sensitization

Mamta Naidu[1], *Rebecca Jones*[1], *Daniel Chang*[1], *Yintak Woo*[1], *Judy Strickland*[2] *and Nicole Kleinstreuer*[3]
[1]Risk Assessment Division/ OPPT/ OCSPP, Environmental Protection Agency, Washington, DC, United States; [2]Integrated Laboratory Systems, RTP, NC, United States; [3]DNTP/ NICEATM, NIEHS/ NIH, RTP, NC, United States
naidu.mamta@epa.gov

The purpose of this project is to develop a framework for assessing the dermal sensitization potential of new or existing chemicals under the Toxic Substances Control Act (TSCA). A clear understanding of structural alerts associated with skin sensitization is needed to recommend appropriate test alternatives to reduce and replace vertebrate animal testing. These tests need to provide information of equivalent (acceptable to OECD guidelines) scientific quality and relevance. Structural associations with local lymph node (LLNA) data were determined by considering physical and chemical properties, *in vivo/in vitro* data, potency and analog data from EPA's Office of Pollution Prevention and Toxics (OPPT) chemical databases and the chemical/structural fragments associated with skin sensitization. We compared these associations with existing structural alerts to determine if skin sensitization can be predicted. For that, chemicals with identified structural alerts will be tested in validated *in vitro* tests for skin sensitization to understand assay applicability domains. Multiple defined approaches and computational models are being considered for integrated testing strategies. A weight of evidence approach (i.e., *in vivo*, *in vitro*, and modeling analyses) will be then used to evaluate skin sensitization potential of identified chemicals.

Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement. Mention of trade names of commercial products should not be interpreted as an endorsement by the U.S. Environmental Protection Agency

II-6-291

Construction of mechanism-based hepatotoxicity prediction system by combining *in silico* and *in vitro* technology

Mizuki Sone, Yuko Nukada, Junko Ohuchi, Shouta Nakagawa, Masayuki Yamane, Naohiro Ikeda and Osamu Morita

Safety Science Research Laboratories, Kao Corporation, Tochigi, Japan

sone.mizuki@kao.co.jp

It is difficult to establish a non-animal evaluation method of systemic toxicity because of its complexity. In this study, we focused on liver which is a main target of repeated dose toxicity test, and we tried to construct the battery methods with *in silico* and *in vitro* to confirm its usefulness for prediction of hepatotoxicity potential.

Three combinations of *in silico* models (HESS, MultiCase, and Derek Nexus) can predict the hepatotoxicity with over 95% sensitivity using 383 chemicals. We evaluated 23 chemicals to confirm a usefulness of *in vitro* assay, which was constructed by several indicators of liver effect, such as cell death, oxidative stress, lipid metabolism, and bile acid accumulation in HepaRG cells. We found that there was no false negative result by combining with *in silico* models and *in vitro* assay. Moreover, *in vitro* assay clarified the mechanisms of the hepatotoxicity and defined the toxic doses to find out the risk by comparing the internal exposure doses *in vivo*.

II-6-419

CRO (contract research organisations) interface to implement and use non-animal new approach methodologies (NAMs) for regulatory purposes. Challenges, opportunities and threats

Costanza Rovida[1] and Monica Locatelli[2]

[1]CAAT-Europe, Konstanz, Germany; [2]TEAM mastery, Como, Italy

costanza.rovida@uni-konstanz.de

Non-animal NAMs are getting interesting to accomplish toxicology needs for regulatory purposes, being faster and eventually more relevant to model the impact of chemical exposure to human health.

One of the difficulties in the expansion of these new approaches is the general lack of experience added to the limited capacity of CRO in offering the service. In fact, most of chemical companies lack internal expertise and labs to run toxicological tests and asks for external support. That's the reason why the role of consultants and CRO is essential for a correct implementation of tests.

Both authors have a long experience in alternative methods applied for Regulatory purposes gained through years of activity in the preparation of REACH (EU Regulation 1907/2006) registration dossiers, working with private companies, CRO and in close collaboration with the Authorities. Even though a tremendous evolution has taken place, a lot of works is still required to improve acceptability and use of NAMs.

References

ECHA (2016). New Approach Methodologies in Regulatory Science. ECHA-16-R-09-EN.

Rovida, C. (2010). Food for thought … why no new in vitro tests will be done for REACH by registrants. *ALTEX 27*, 175-183.

Rovida, C., Alépée, N., Api, A. M. et al. (2015). Integrated Testing Strategies (ITS) for safety assessment. *ALTEX 32*, 25-40.

II-6-634

Ways to address repeated-dose systemic toxicity: Past and future

Reine Note

L'Oréal Research & Innovation, Paris, France

rnote@rd.loreal.com

The L'Oréal laboratories have started working on alternative methods to animal testing for decades. They have continued strengthening their expertise through international collaborations to develop new approaches, particularly for acute and repeated-dose systemic toxicity. Results obtained from two major projects in these fields with private institutions and the US EPA, demonstrate the importance of considering the specificity of cosmetic ingredients in terms of chemical reactivity and classes of solubility profiles. Lessons learned also include the need for proceeding in a step-wise manner, starting from basic and streamlined models (e.g. targeted organ toxicity and mechanism of action) to more sophisticated ones, like omics-based and systems biology models integrating kinetics and the dynamics of the responses. Herein, we will show that such considerations drive our industry to continuous efforts towards accurate safety assessment of new ingredients.

II-6-800

The emerging utility of human IPS cell-derived cardiomyocytes in evaluation of proarrhythmic risk

Derek Leishman

Global PKPD & Pharmacometrics, Eli Lilly & Company, Indianapolis, IN, United States

Derek.leishman@lilly.com

Testing for a new medicine's potential to prolong cardiac repolarization and cause the arrhythmia torsade de pointes has been an area of focus for the pharmaceutical industry for two decades now. It also has its own international testing guidelines for both clinical and nonclinical testing. While regulatory points to consider no longer suggest a study in a canine Purkinje fiber preparation a recent industry survey suggests that most pharmaceutical companies are using additional preclinical studies to characterize molecules. Alongside the testing which has occurred for new molecules, the last twenty years have contributed to the understanding of the mechanistic underpinnings of the issue. Recently this has contributed to a new regulatory initiative to utilize *in silico* models and human IPS cell-derived cardiomyocytes to replace the existing paradigm. The presentation will describe the historical background, the new initiative and measures of utility for this alternative method. The learnings from this relatively well understood issue can potentially be applied to other nonclinical safety issues.

II-6-672

Development of a framework for intelligent evaluation of non-animal methods for safety assessments

Raja Settivari[1] and HESI Framework for Intelligent Non-Animal Methods[2]

[1]Toxicology & Environmental Research & Consulting, Dow Chemical Company, Midland, MI, United States; [2]ILSI Health and Environmental Sciences Institute (HESI), Washington, DC, United States
rssettivari@dow.com

A coordinated effort is needed to increase scientific confidence in the utility and interpretation of new alternative methods (NAMs) for pharmaceutical and chemical safety and to establish their fit-for-purpose for regulatory safety assessment. ILSI-HESI launched a project in 2014 bringing together experts from government, industry, non-governmental organizations, and academia to determine and integrate criteria for assessing fitness-for-purpose. A framework was created along with its application through case studies for ocular irritation, dermal and respiratory sensitization, cardiac safety and developmental vascularization. This will help increase confidence in NAMs and will provide guidance on development of regulatory or organization-specific guidelines for their application and interpretation in safety assessment and regulatory decision making.

Session II-7: Novel Uses of Genomics for Product Safety

Co-Chairs
Roland Grafstrom, Karolinska Institute, Stockholm, Sweden
George Daston, Procter & Gamble, Cincinnati, OH, United States

II-7-338

A predictive toxicogenomics space-based scoring tool provides novel means of applying genomics to product safety

Roland Grafstrom, Penny Nymark and Pekka Kohonen

Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden
roland.grafstrom@ki.se

Mechanistic analyses of toxicity pathways based on "omics" are key to an emerging *in-vitro*-only-based safety assessment and for identifying Adverse Outcome Pathways (AOPs). We applied component modeling of the gene expression space in the Connectivity Map versus toxicity data, and generated a "predictive toxicogenomics space (PTGS)"-based scoring tool that captures dose-dependent toxicity of diverse agents. The scoring generates a probability estimate intrinsic to any transcriptomics data via broad coverage of cellular toxicity mechanisms. The tool predicts liver injury in animals (long-term repeated-dose toxicity bioassays) from hepatocyte experiments, covering all known pathologies in the TG-GATEs database. Dose levels of concern for drug-induced liver injury in humans are also predicted by PTGS, serving complementary relative other prediction assays. Overall, "omics" data application for toxicity modeling and AOP exploration *in vitro* is effectively demonstrated by the PTGS concept.

References

Grafström, R. C., Nymark, P., Hongisto, V. et al. (2015). Toward the replacement of animal experiments through the bioinformatics-driven analysis of 'omics' data from human cell cultures. *Altern Lab Anim 43*, 325-332.

Kohonen, P., Ceder, R., Smit, I. et al. (2014). Cancer biology, toxicology and alternative methods development go hand-in-hand. *Basic Clin Pharmacol Toxicol 115*, 50-58.

Kohonen, P., Parkkinen, J. A., Willighagen, E. L. et al. (2017). A transcriptomics data-driven gene space accurately predicts liver cytopathology and drug-induced liver injury. *Nat Commun 8*, 15932.

II-7-495

Industry approaches for botanical safety evaluation, without the use of animals

Karen Vandermoeln, Catherine Mahony, Jorge Naciff and George Daston

Procter and Gamble, Cincinnati, OH, United States
vandermolen.k@pg.com

As complex mixtures, botanicals present unique challenges when assessing safe use, particularly when endpoint gaps exist that cannot be fully resolved by existing toxicological literature. Obtaining data for genotoxicity and developmental and reproductive toxicity can be particularly difficult, and so a weight of evidence strategy for these endpoints will be illustrated which utilizes new *in vitro* approaches. Both receptor binding assays and gene expression studies have been explored as tools to inform on modes of action and active components. Several extracts of both food herbs and botanicals known to have reproductive effects were tested against a suite of receptors at biologically relevant doses to probe developmental and reproductive activity. Gene expression changes were observed in a proof-of-concept study, and has shown utility in identifying major active components.

II-7-336

A Predictive Toxicogenomics Space (PTGS)-based concept and gene set enrichment analysis serves sensitively to benchmark dose (BMD) evaluation of cytotoxicity effects

Pekka Kohonen, Penny Nymark and Roland Grafström
Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
pekka.kohonen@ki.se

Single sample gene set enrichment analysis (Hänzelmann et al., 2013) was applied to a recently developed "predictive toxicogenomics space (PTGS)" concept (Kohonen et al., 2014, 2017; Grafström et al., 2015) for performing BMD analysis *in vitro*. Tumor suppressor P53-related endpoints were analyzed, applying dose-response transcriptomics data of influences of etoposide, methyl methanesulfonate or quercetin in HT1080 cells (Clewell et al., 2014). We found that the PTGS-based transcriptomics BMDLs (5% lower limit of the BMD) identified toxicity-related points of departures (PODs) as soon as there was any change in gene expression. Overall, these PODs were up to 100-fold more sensitive than p53 target gene-based transcriptomic PODs identified by the commonly applied U.S. EPA BMDExpress software (Kuo et al., 2016; Andersen et al., 2015). Moreover, the PTGS-based BMDLs were equally dose sensitive as micronucleus formation, being the most sensitive endpoint in the original study. Validation of the PTGS concept for quantitative dose-dependent BMD modeling *in vitro* is directly relevant to the wish of applying omics data into 3R research and toxicity testing, ultimately aiming for a full replacement of animal experimentations.

References

Andersen, M. E., McMullen, P. D. and Krewski, D. (2015). Developing tools for defining and establishing pathways of toxicity. *Arch Toxicol 89*, 809-812.

Clewell, R. A., Sun, B., Adeleye, Y. et al. (2014). Profiling dose-dependent activation of p53-mediated signaling pathways by chemicals with distinct mechanisms of DNA damage. *Toxicol Sci 142*, 56-73.

Grafström, R. C., Nymark, P., Hongisto, V. et al. (2015). Toward the replacement of animal experiments through the bioinformatics-driven analysis of 'omics' data from human cell cultures. *Altern Lab Anim 43*, 325-332.

Hänzelmann, S. Castelo, R. and Guinney, J. (2013). GSVA: Gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics 14*, 7.

Kohonen, P., Ceder, R., Smit, I. et al. (2014). Cancer biology, toxicology and alternative methods development go hand-in-hand. *Basic Clin Pharmacol Toxicol 115*, 50-58.

Kohonen, P., Parkkinen, J. A., Willighagen, E. L. et al. (2017). A transcriptomics data-driven gene space accurately predicts liver cytopathology and drug-induced liver injury. *Nat Commun 8,* 15932.

Kuo, B., Francina Webster, A., Thomas, R. S., Yauk, C. L. et al. (2016). BMDExpress Data Viewer – a visualization tool to analyze BMDExpress datasets. *J Appl Toxicol 36*, 1048-1059.

II-7-108

HIPPTox: High-throughput *in vitro* phenotypic profiling for toxicity prediction

Lit-Hsin Loo[1] and Daniele Zink[2]
[1]Bioinformatics Institute, Agency for Science, Technology and Research, Singapore; [2]Institute of Bioengineering and Nanotechnology, Agency for Science, Technology and Research, Singapore
loolh@bii.a-star.edu.sg

Chemicals originating from medicine, food, or the environment may disrupt cellular functions and cause adverse changes in cellular phenotypes. *In vitro* human cell-based models have the potential to replace animal models for detecting these effects. However, most existing *in vitro* models are based on cell death/viability end points, which have been repeatedly shown to be poorly predictive of *in vivo* toxicity. I will present our recent work in developing "High-throughput In-vitro Phenotypic Profiling for Toxicity prediction" (HIPPTox) Platform, which can be used to automatically identify quantitative and predictive *in vitro* toxicity endpoints from microscopy images of cells exposed to large numbers of reference toxic compounds. We have applied the platform to build *in vitro* human cell-based and computational models that are highly predictive of human nephrotoxicity[1], and more recently of pulmonary toxicity. These models may be used as high-throughput alternatives for assessment chemical hazards.

Reference

Su, R., Xiong, S., Zink, D. and Loo, L.-H. (2016). High-throughput imaging-based nephrotoxicity prediction for xenobiotics with diverse chemical structures. *Arch Toxicol 90*, 2793-2808.

II-7-88

Omics-based *in vitro* verification of an adverse outcome pathway of cholestatic liver injury

Mathieu Vinken[1], Robim M. Rodrigues[1], Laxmikanth Kollipara[2], Umesh Chaudhari[3], Agapios Sachinidis[3], René P. Zahedi[2], Albert Sickmann[2], Annette Kopp-Schneider[4], Xiaoqi Jiang[4], Hector Keun[5], Jan Hengstler[6], Tineke Vanhalewyn[1], Joery De Kock[1], Tamara Vanhaecke[1] and Vera Rogiers[1]

[1]In Vitro Toxicology, Vrije Universiteit Brussel, Brussels, Belgium; [2]Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany; [3]Institute of Neurophysiology and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany; [4]Division of Biostatistics, German Cancer Research Center, Heidelberg, Germany; [5]Computational and Systems Medicine, Department of Surgery and Cancer, Imperial College London, London, United Kingdom; [6]Leibniz Research Centre for Working Environment and Human Factors, Technical University of Dortmund, Dortmund, Germany

mvinken@vub.ac.be

An adverse outcome pathway (AOP) construct has been previously introduced to pinpoint the mechanisms in drug-induced cholestasis. The molecular initiating event in this AOP is the inhibition of the bile salt export pump (BSEP), while the key events that are subsequently triggered include bile accumulation, induction of oxidative stress and inflammation, cell death and the activation of specific nuclear receptors. The present study was set up to evaluate the reliability of this AOP for cholestatic liver injury and to come up with new biomarkers that support its key events. For this purpose, human hepatoma-derived HepaRG cells were exposed to subcytotoxic concentrations of bosentan, a potent BSEP inhibitor, known to clinically induce cholestasis. The cellular response to the inflicted toxicity was evaluated by means of transcriptomics, proteomics and metabolomics techniques. Pathway analysis of both transcriptomics and proteomics data identified cholestasis as a major toxicological event. Transcriptomics results further showed several of the predicted gene changes related to the activation of nuclear receptors. Induction of oxidative stress was also observed. Furthermore, 37 genes could be identified by microarray analysis of samples of cells exposed to all tested concentrations of bosentan. Of those, 10 were also significantly modified at the protein level. These could be proposed as potential novel transcriptional biomarkers of bosentan-induced cholestasis. Metabolomics analysis indicated changes in specific endogenous metabolites related to mitochondrial impairment. The outcome of this study underscores the scientific soundness of the previously established AOP of cholestasis and demonstrates the power of *in vitro* testing for optimizing AOPs.

II-7-676

Deriving pathways of toxicity from -omics data: Endocrine disruptors as a case study

Alexandra Maertens, Andre Kleensang and Thomas Hartung

Center for Alternatives to Animal Testing, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

amaerte1@jhu.edu

Systems toxicology has transformed toxicology from a reductionist approach to a more high-level view that takes advantage of the newer, highcontent and highthroughput technologies. From a hazard assessment perspective, it offers an opportunity to move away from the limited mechanistic information provided by a traditional "blackbox" animal tests to a pathway based approach that can provide a detailed mechanistic understanding at a cellular level. Endocrine disruptors have proven very difficult to understand using animal models, as they have subtle effects over a long-term exposure and not necessarily with a monotonic doseresponse curve. Here, we show how using a network based approach to an *in vitro* microarray dataset of endocrine disruptors over both a doseresponse curve and a timecourse can derive a basic Pathway of Toxicity and better elucidate the complexity of cellular responses to estrogenic substances. Moreover, a network approach to *in vitro* data can help identify the possible thresholds of concern, and generate candidate biomarkers for adverse outcomes.

Session II-8: Tipping Point – Using *In Vitro* to Discriminate Adverse from Adaptive Effects

Co-Chairs
Bob Van De Water, Leiden University, The Netherlands
Shuangqing Peng, Institute of Disease Control and Prevention, AMMS, Beijing, People's Republic of China

II-8-514

Systems biology modelling of adaptive cellular stress response signalling in chemical safety assessment

Bob van de Water

Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

b.water@lacdr.leidenuniv.nl

To increase our understanding of chemically-induced adaptive stress response pathway activation and its contribution to safety assessment a time-resolved, sensitive and multiplex readout of chemical-induced toxicological relevant cellular stress responses is essential. For this we developed a platform containing a wide panel of distinct adaptive stress response fluorescent protein reporter cell lines, based on BAC-GFP transgenomics approaches thus conserving the endogenous gene regulatory elements. We have tagged key regulatory genes of oxidative stress response, unfolded protein response and DNA damage response. These are used for automated high content live cell imaging and quantitative multi-parameter image analysis of pathway activation. Here we demonstrate the functionality and application of individual BAC-GFP reporters to systematically monitor and map the activation of the Nrf2 signaling pathway using automated imaging. We have applied the quantitative dynamics information of KEAP1, Nrf2 as well as the downstream Nrf2 target gene Srxn1 to establish systems biology ODE-based mathematical models to predict Nrf2 pathway activation.

II-8-732

Finding toxicological tipping points from high-content imaging data

Imran Shah

National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park (RTP), NC, United States

shah.imran@epa.gov

A key challenge to using *in vitro* data in risk assessment is differentiating between chemical-induced adaptive versus adverse cellular responses. To further investigate this issue, we studied the effects of hundreds of chemicals in HepG2 cells using high-content imaging (HCI). HCI measured chemical concentration and time-dependent perturbations in p53, JNK, oxidative stress, cytoskeleton, mitochondria, and cell cycle progression. We developed a novel computational model to analyze these multidimensional HCI datastreams, and used this model to interpret the dynamic responses to chemicals as cell-state trajectories. By analyzing trajectories for each chemical, we found three concentration-dependent trends in HepG2 cell behaviors including: (a) adaptation followed by complete recovery, (b) adaptation followed by partial recovery, and (c) adaptation without recovery leading to irreversible injury. We consider the concentration-dependent transition from adaptation to injury a "tipping point" of the system. Using Boolean network (BN) reconstruction to systematically analyze all trajectories, we found putative regulatory processes that may explain the basis of cellular resilience. We believe that multidimensional and dynamic *in vitro* data for chemicals can be interpreted with computational models to find tipping points, and gain unique mechanistic insight into the threshold for homeostatic adaptation. With additional work, tipping points could also be used as the point of departure for risk assessment.

This abstract does not necessarily reflect U.S. EPA policy.

II-8-292

Towards toxicity pathway-based risk assessments with a case study on doxorubicin: Bridging *in vitro* tipping points with *in vivo* human clinical risk using PBPK modelling

Shuangqing Peng[1], Haitao Yuan[1], Qiang Zhang[2], Hequn Li[3], Jiabin Guo[1], Ian Sorrel[3], Alistair Middleton[3], Jin Li[3], Paul. L Carmichael[3] and Carl Westmoreland[3]

[1]Evaluation and Research Centre for Toxicology, Institute of Disease Control and Prevention, Academy of Military Medical Sciences, Beijing, People's Republic of China; [2]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, United States; [3]Unilever Safety and Environmental Assurance Center, Bedford, United Kingdom

pengsq@hotmail.com

Chemical safety assessment is undergoing a paradigm shift from the traditional animal-based toxicity testing methods to novel, human cells-relevant, toxicity pathway-based *in vitro* approaches, coupled with *in silico* models. One of the key challenges is how information gleaned from relevant cell assays, for example toxicity pathway-perturbing concentrations of chemicals (i.e., *in vitro* tipping points), can be used to inform the risk of human health in an appropriate way.

A chemotherapy drug doxorubicin (DOX) was chosen as a prototype chemical in this study for its well-known cardiotoxicity in patients. An *in vitro* tipping point of 125 nM for a single 12-hour exposure was previously identified in the AC16 human cardiomyocytes by using an integrated experimental and computational modelling approach based on pathway perturbation of proliferator-activated receptor γ coactivator 1α (PGC-1α)-mediated transcriptional network (Yuan et al., 2016). A similar tipping point, around 156 nM, was also demonstrated lately in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), where a single 2-day exposure did not lead to any functional adverse effects in these cells, whilst repeated DOX exposure over a 6-day period resulted in long-term arrhythmic beating accompanied by significant cytotoxicity (Chaudhari et al., 2016).

In order to understand the significance of the different *in vitro* experimental scenarios in informing human risk in clinic, we have carried out substantial literature review on Dox in human clinical studies. Four *in vivo* doses with different clinical symptoms from no adverse effects observed, adverse cardiotoxicity effects observed in few cases, to chronic toxicity-CHF observed, were selected to benchmark against *in vitro* tipping points. They are 1) 5 mg/m^2/day continuous i.v. infusion with no adverse effects observed; 2) 9 mg/m^2/day 30-min i.v. infusion with adverse effects observed in very few patients; 3) 9 mg/m^2/day continuous i.v. infusion with few adverse cases; 4) 30 mg/m^2/day 30-min i.v. infusion with chronic toxicity- Congestive Heart Failure (CHF) observed in patients.

A human population-based PBPK model were developed properly and verified further using some human clinical measurements. When applying PBPK modelling to bridge *in vitro* tipping points with *in vivo* clinical doses, we assumed *in vitro* tipping point concentrations be reached at steady-state levels either in human blood plasma or in hearts. Our comparisons were conducted based on two distinctive PBPK metrics: C_{max} (the maximum concentration) and Area Under the Curve (AUC) where exposure periods play an essential part in calculation, like the applied doses. Our comparison results imply that a) when applying the PBPK modelling-facilitated reverse dosimetry approach, the assumed heart-targeted concentrations are more risk-averse than the assumed plasma-targeted levels in terms of protecting human. b) AUC-based metrics that take exposure into consideration were more conservative than C_{max}-based in terms of human safety assessments. In general, when an adequate PBPK model is employed and a proper metric is applied, the *in vitro* tipping point is capable of informing human cardiovascular risk of Dox to a large extent. In addition, our study has also enabled us to find a few potential uncertainty factors in our study which should be considered in our future research, including *in vitro* free concentrations kinetics, active transporters and metabolism, *in vivo* intracellular and extracellular concentrations in organs, etc.

Our case study on Dox illustrated that *in vitro* tipping points do show some merits of indicating human clinical risk when an adequate reverse dosimetry PBPK modelling is applied and a proper PBPK metric is used. The framework demonstrated in this study could pave a way to reduce the need of animal testing for human risk assessment.

References

Chaudhari, U., Nemade, H., Wagh, V. et al. (2016). Identification of genomic biomarkers for anthracycline-induced cardiotoxicity in human iPSC-derived cardiomyocytes: An in vitro repeated exposure toxicity approach for safety assessment. *Arch Toxicol 90*, 2763-2777.

Yuan, H., Zhang, Q., Guo, J. et al. (2016). A PGC-1alpha-mediated transcriptional network maintains mitochondrial redox and bioenergetic homeostasis against doxorubicin-induced toxicity in human cardiomyocytes: Implementation of TT21C. *Toxicol Sci 150*, 400-417.

II-8-198

Correlation of *in vitro* cytotoxicity and acute toxicity

Judy Strickland[1], Agnes Karmaus[1], Xiaoqing Chang[1], Mike Paris[1], Dave Allen[1], Nicole Kleinstreuer[2] and Warren Casey[2]

[1]ILS, Morrisville, NC, United States; [2]NIH/NIEHS/DNTP/NICEATM, RTP, NC, United States

jstrickland@ils-inc.com

Although cytotoxicity assay data cannot currently be used to replace animal tests for predicting acute hazard classes, two *in vitro* cytotoxicity assays have been validated to estimate starting doses for acute oral toxicity tests in animals. To more broadly investigate the utility of cytotoxicity and other *in vitro* assays to predict acute lethality, high throughput screening (HTS) data from the ToxCast and Tox21 programs was used to predict LD_{50} values and binary toxicity categories (toxic vs. nontoxic). To further investigate the correlation of *in vitro* to *in vivo* results, we used reverse toxicokinetics to estimate equivalent administered doses from *in vitro* effective concentrations. These analyses confirmed that no single *in vitro* assay can currently predict acute systemic toxicity in rodents and prompted us to evaluate approaches that combine assay results.

This work was supported with U.S. Federal funds from NIEHS/NIH/HHS under Contract HHSN273201500010C.

Theme II: Lessons Learned

Poster Presentations

II-30

Me-too validation study of reconstructed human corneal epithelial model, LabCyte CORNEA-MODEL24 eye irritation test method

Hidefumi Ikeda[1], Satoshi Nakahara[2], Hajime Kojima[3], Takashi Omori[4], Mai Endo[5], Maiko Satake[5], Saori Hagiwara[6], Shinsuke Shinoda[6], Hideki Nishiura[7], Toshihiko Kasahara[8], Yusuke Yamamoto[8], Masakazu Katoh[9] and Katsura Sugawara[9]

[1]Mandom Corporation, Osaka, Japan; [2]Maruishilabo Corporation, Osaka, Japan; [3]National Institute of Health Sciences, Tokyo, Japan; [4]Kobe University, Hyogo, Japan; [5]Doshisha University, Kyoto, Japan; [6]Drug Safety Testing Center Co., Ltd, Saitama, Japan; [7]Nihon Kolmar Co., Ltd, Osaka, Japan; [8]Fuji Film Corporation, Kanagawa, Japan; [9]Japan Tissue Engineering Co., Ltd, Aichi, Japan
ikeda-hi@mandom.co.jp

New eye irritation test (EIT) method using reconstructed human corneal epithelial (RhCE) model, LabCyte CORNEA-MODEL24 (LabCyte24), had established through several pre-validation studies. From the assay principle of LabCyte24 EIT is similar to the RhCE EIT for the OECD test guideline 492 (TG492), the me-too validation study of LabCyte24 EIT was planned according to the performance standard (PS) for TG492. Three participating laboratories performed 3 independent runs for 30 test chemicals listed in the PS. Within- and between-laboratory reproducibility rate were from 93% to 100% and 90% and therefore target values (≥ 90% and ≥ 85%) in the PS were achieved. It was also demonstrated good predictive capacity with accuracy from 83% to 84%, sensitivity from 93% to 100%, and specificity from 66% to 73%, thereby meeting the acceptance criteria (≥ 75%, ≥ 90%, and ≥ 60%) stipulated in the PS. These results provide information useful to propose LabCyte24 EIT as me-too method for inclusion in TG492.

II-36

Retinoic acid as an example for a toxicokinetic study in a multi organ chip comprising EpiDerm skin models and liver organoids

Andreas Schepky[1], Ilka Maschmeyer[2], Thamée Rings[1], Juliane Huebner[2], Silke Gerlach[1], Uwe Marx[2] and Jochen Kuehnl[1]

[1]Department Global Toxicology, Beiersdorf AG, Hamburg, Germany; [2]TissUse GmbH, Berlin, Germany
andreas.schepky@beiersdorf.com

For cosmetics industry, the current lack of *in vitro* assays for repeated subacute toxicity is a bottleneck for innovation, since safety assessment relies exclusively on alternatives to animal approaches. Dynamic organ-on-chip cell culture technologies are expected to provide future *in vitro* testing options. Hence, we aimed to evaluate the ability of TissUse's two-organ-chip (2-OC) technology to provide relevant information on compound metabolism and effects on gene expression after repeated, long-term, dosing regimens. To this end, we performed and show a successful case study with all-trans retinoic acid (ATRA) in an integrated system comprising EpiDerm™ skin models and 3D liver organoids. Hence, multi-organ-chip-based toxicokinetic approaches may add important aspects such as skin barrier function, metabolism, potentially, organ-organ-interactions, and additionally toxicodynamics (albumin, LDA) and could be a valuable tool for future safety assessment without animal testing.

Reference

Maschmeyer, I., Lorenz, A. K., Schimek, K. et al. (2015). A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip 15*, 2688-2699.

II-53

Introducing optical coherence tomography for organotypic retina cultures

Sven Schnichels, José Hurst, Karl Ulrich Bartz-Schmidt, Kai Januschowski and Focke Ziemssen

University Eye Hospital Tübingen, Tübingen, Germany
sven.schnichels@med.uni-tuebingen.de

Optical coherence tomography (OCT) dramatically changed the way of diagnostic assessment in retinal diseases. Since, organotypic retinal cultures are continuously replacing *in-vivo* experiments in ophthalmic research, we adapted OCT-measurements to retinal cultures. An easy to use protocol was generated to assess standardized OCT assessments: Only two custom-made devices are needed to change any commercially available OCT for examinations in humans into a device allowing *ex-vivo* analyses of organotypic retinal cultures. The modification is feasible within seconds. OCT pictures of *ex-vivo* retinas were obtained for time periods of up to seven days and the thickness of retinal tissue was evaluated via ImageJ software. The reproducibility of the pictures and measurements was very high (SD < 15%).

In conclusion, an easy to use protocol for the investigation of different effects on retinal cultures with commercially available OCT devices was successfully established.

II-87

Development and evaluation of kanamycin loaded lipid modified DNA-nanoparticles via *ex vivo* infection models

Jose Hurst[1], Marina Löscher[1], Jan Willem de Vries[1], Karl-Ulrich Bartz-Schmidt[1], Martin Spitzer[2] and Sven Schnichels[2]

[1]University Eye Hospital Tübingen, Tübingen, Germany; [2]Eye Clinic, University Hospital Hamburg-Eppendorf, Hamburg, Germany
jose.hurst273@gmail.com

Purpose: We generated lipid-modified DNA-nanoparticles (NPs), which allow improved adherence to the eye surface and can therefore be used as a vehicle for enhanced delivery of eye medication. To treat cornea infections, we tested kanamycin-loaded NPs (Kan-NP) and evaluated them on two *ex-vivo* cornea infection models.

Methods: Ex-vivo pig corneas were treated with Kan-NP, incubated with *E. coli* or *P. aeruginosa* and afterwards the number of bacteria was determined. To validate the models the experiments were also conducted *in-vivo*.

Results: Kanamycin and Kan-NPs were able to significantly decrease the amount of *E. coli* and *P. aeruginosa* colonies compared to the control. Our Kan-NP were more efficient than the free kanamycin as confirmed in the *in-vivo* study.

Conclusions: Both *ex-vivo* infection models are suitable for evaluating new treatments. After proving the *in-vivo* like situation of the *ex-vivo* models, new antibiotic eye-drops can now be easily tested with these *ex-vivo* models.

II-92

Successful incorporation of alternative method programs for diverse toxicology applications at a large corporation

Lawrence Milchak, David Brandwein and Colin Owens

Corporate Medical Department, 3M, St. Paul, MN, United States
lmilchak@mmm.com

3M has always been firmly committed to animal use reduction and over the last ten years, significant progress has been made at utilizing alternative methods for evaluating human health hazard potential of raw materials and products. A particular challenge is finding methods to effectively work across a broad product portfolio, including industrial, consumer and health care products, while having cost effective utility for product development. These efforts at 3M have primarily been focused on building an internal computational toxicology program and developing alternative test method capabilities within the 3M Strategic Toxicology Laboratory (STL), an internal resource providing toxicology support to 3M businesses. A focus in the STL has been on use of reconstructed human three-dimensional (3D) tissues. These commercially available tissues are much more effective for evaluating mixtures, formulations and finished products for irritation and toxicity potential, compared to standard two-dimensional cell culture methods, particularly when the test materials are often insoluble in cell culture media. Numerous 3D human tissue types have been successfully used in the STL, including dermal, ocular, oral and vaginal models, while also utilizing unique delivery systems for vapor/gas exposures to airway tissues. Standardized assays, such as OECD 431, 439 and 492 have been utilized for a variety of purposes, including hazard identification and safety assessments, hazard communication, new chemical registrations and transportation classifications. Time course assays have also proven very useful for formulation comparison and product development purposes, while other customized studies have evaluated the efficacy of various skin washing agents at removal of coating products from the skin to formulate occupational health recommendations for workers, and skin protection assays to investigate the efficacy of barrier products for health care applications. The computational program utilizes multiple approaches in evaluations for read-across, structural alerts and (Q)SAR analysis to build a weight of evidence approach for preparing toxicity estimates. Customized (Q)SAR models utilizing historical test data have been built for specific endpoints of interest, such as inhalation toxicity. A database has also been built to store the computational results that is searchable by chemical structure/similarity and currently contains over 1000 chemicals that have been assessed. The results of these alternative efforts have provided valuable information for safety and risk assessment purposes for highly diverse products, particularly during product development, while allowing significant reductions in animal use. A key learning has been ensuring that the alternative methods incorporated have practical utility to the business needs and the product types to be evaluated. Integration and coordination of the alternative assessment processes has also been found to be critical to maximize their effectiveness.

II-94

Non-animal testing strategy for skin sensitization assessment of hydrophilic and lipophilic chemicals

Hideyuki Mizumachi, Yuki Otsubo, Taku Nishijo, Kazutoshi Saito, Masaaki Miyazawa and Hitoshi Sakaguchi

Kao Corporation, Tochigi, Japan
mizumachi.hideyuki@kao.co.jp

Integrated testing strategies (ITS) using multiple non-animal test methods have been developed to predict chemical sensitizing potential. A negative result in the DPRA, KeratinoSens and h-CLAT methods predict chemical to be a non-sensitizer due to a lower false negative prediction compared with LLNA. While one positive result in any of the three tests suggests chemical to be a sensitizer with Bayesian Network ITS-3 providing the quantitative weight of evidence for potency prediction. But, lipophilic chemicals (e.g. log Kow ≥ 3.5) may result in a false prediction due to technical limitations. To address this issue, we developed a non-animal test method, EpiSensA, using reconstructed human epidermis. Existing and newly generated data for more than 50 lipophilic chemicals resulted in a high predictive performance with EpiSensA compared to the three tests. These findings show a practical workflow of skin sensitization evaluation using multiple ITS for hydrophilic and lipophilic chemicals.

II-129

Non-animal testing approach to address biocompatibility testing of medical devices required by the United States Food and Drug Administration (US FDA)

Erin Hill and Emilia Costin

Institute for In Vitro Sciences, Gaithersburg, MD, United States
ehill@iivs.org

Starting in December 2015, personal lubricants must receive pre-market approval from the US FDA Center for Devices and Radiological Health (CDRH) in order to be sold in the US. Part of the testing battery for biocompatibility includes the *in vivo* Rabbit Vaginal Irritation (RVI) test. We have created an Industry Consortium comprised of personal lubricants manufacturers and are working collaboratively with stakeholders and the US FDA to develop an *in vitro* testing approach to substitute for the RVI. Our Validation Program will analyze paired *in vivo* (and/or clinical)-*in vitro* data for vaginal irritation utilizing commercially available human reconstructed vaginal tissue models. A prediction model will be proposed that can be used for the safety assessment of personal lubricants. Our Validation Program proposal has been accepted in the Incubator Phase of the US FDA Medical Device Development Tool (MDDT) Pilot Program and is currently ongoing.

II-147

In vitro evaluation of epichlorohydrin nanostructured chitosan scaffolds: OECD adapted method

Patricia Lopes[1], Julia Ernesto[1], Gabriela Pessoa[1], Classius Silva[2] and Monica Mathor[3]

[1]Departamento de Ciências Farmacêuticas, Universidade Federal de São Paulo (UNIFESP), Diadema, Brazil; [2]Departamento de Engenharia Química, Universidade Federal de São Paulo (UNIFESP), Diadema, Brazil; [3]Centro de Tecnologia em Radiações, Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN SP), São Paulo, Brazil
patricia.lopes@unifesp.br

Tissue bioengineering can be defined as a tool to guided regeneration of tissue using materials that serve as templates for ingrowth of host cells. The ISO 10993 describes the tests that must be performed to assess the biocompatibility of these new materials. Although the ISO recommends tests performed *in vitro* based on OECD guides, in order to apply these assays further validation for this specific area is essential. The aim of this work was to prepare and evaluate Chitosan 2% (w/v) as a biomaterial crosslinked with epichlorohydrin (0.01 mol/L) that results in a nanostructured material with interconnected pores showed by scanning electron microscopy and with adhesion properties (DAPI test). Cytotoxicity, phototoxicity and genotoxicity tests were adapted and carried out and they showed that the scaffolds do not present cytotoxic, phototoxic or genotoxic potential allowing the tests to be continued to assess its use as a dressing to enhance the healing process in severe skin lesions.

References

ISO 10993-5 – International Organization for Standardization (2009). Biological evaluation of medical devices – Part 5: Tests for cytotoxicity: In vitro methods. Switzerland.

ISO 10993-12 (2012). Biological evaluation of medical devices – Part 12: Sample preparation and reference materials. Switzerland.

OECD (2004). Guideline for Testing of Chemicals 432: In vitro e 3T3 NRU phototoxicity test.

OECD (2014). Guideline for Testing of Chemicals 487: In Vitro Mammalian Cell Micronucleus Test (MNvit).

II-251

Development of an animal product free acute toxicity screen

Christopher Longmore, Nathalie Belot, Carol Treasure, Bushra Sim and Lottie Roscoe

XCellR8 Ltd, Daresbury, United Kingdom
chris.longmore@x-cellr8.com

Acute toxicity is a key human health endpoint, requiring assessment by many global chemical safety regulators. Despite concerns on both ethical and scientific grounds, animal-based methods are used for acute toxicity with no validated alternative available or in development. XCellR8 has developed an acute toxicity screen using animal-product-free conditions. Comprised of a modified neutral red uptake (NRU) method and novel prediction model, we have screened 20 cosmetic ingredients to produce IC_{50} values (concentration where cell viability is 50%). Through analysis of IC_{50} values with the prediction model, 13/15 blind-tested chemicals were placed into the same GHS category as that derived from *in vivo* acute toxicity studies. This *in vitro* acute toxicity test is proposed as the initial step of an IATA, incorporating multiple organ-specific endpoints. XCellR8 next hopes to model metabolism in the current test and promote its utility for other chemical sectors.

References

Barker-Treasure, C., Coll, K., Belot, N. et al. (2015). Non-animal replacements for acute toxicity testing. *Altern Lab Anim 43*, 199-203.

EURL ECVAM (2017). Acute Toxicity. https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/systemic-toxicity/acute-toxicity#background (accessed 30 March 2017).

Hoffmann, S., Kinsner-Ovaskainen, A., Prieto, P. et al. (2010). Acute oral toxicity: Variability, reliability, relevance and interspecies comparison of rodent LD_{50} data from literature surveyed for the AcuteTox project. *Regul Toxicol Pharmacol 58*, 395-407.

II-273

Better understanding of bioavailability of cosmetic ingredients: Results from Cosmetics Europe skin bioavailability project

Andreas Schepky[1], Richard Cubberley[2], Hélène Duplan[3], Joan Eilstein[4], Corie Ellison[5], Sébastian Gregoire[4], Nicola Hewitt[6], Carine Jacques-Jamin[3], Daniela Lange[1], Amy Roe[5], Helga Rothe[7] and Martina Klaric[6]

[1]Beiersdorf AG, Hamburg, Germany; [2]Unilever, Bedford, United Kingdom; [3]Pierre Fabre Dermo-Cosmétique, Castres, France; [4]L'Oréal, Paris, France; [5]Procter & Gamble, Cincinnati, OH, United States; [6]Cosmetics Europe, Brussels, Belgium; [7]Coty, Darmstadt, Germany
andreas.schepky@beiersdorf.com

Due to the animal testing ban for cosmetics, the Cosmetics Europe Skin Bioavailability and Metabolism Task Force was set up to improve existing methods and develop new tools to measure and predict skin bioavailability of cosmetic ingredients. Eight assays were conducted under standardised conditions (including skin penetration and metabolism, partition/diffusion coefficients in different skin layers and peptide binding) to allow comparison across chemicals and improvement of *in silico* skin penetration models. In a second step, these assays were used to determine the fate of 50 chemicals after application to the skin. Results provide relevant and standardized information on the local skin and systemic concentrations of chemicals and can be used in combination with PBPK models, cheminformatics and AOPs to refine the assessment of local and systemic toxicity of chemicals applied to the skin. Results of up to 50 chemicals will be presented and discussed.

II-281

The human Cell Line Activation Test (h-CLAT) for assessment of dermal sensitization potency of commercially available mixtures and the OECD proficiency chemicals

Bennett Varsho, Micheal Carathers, Puneet Vij and George DeGeorge

MB Research Labs, Spinnerstown, PN, United States
bvarsho@mbresearch.com

To explore the applicability domain of the h-CLAT beyond pure chemicals and into mixtures, we analyzed THP-1 cell expression of CD86 and CD54 cell surface markers from an expanded set of OECD proficiency chemicals and several mixtures. The h-CLAT correctly predicted 14 of 15 of these chemicals, an Accuracy of 93.7%. A variety of products from the petroleum, agrochemical, food, cosmetic and chemical industries were obtained via retail outlets and evaluated, including: PPD and non-PPD based hair dyes, propolis extract, fuel additives, pesticides, and adhesives. The h-CLAT correctly predicted the sensitization properties of these mixtures with an accuracy of 91%. Thus, we show the feasibility of the h-CLAT to determine the sensitization potential for both pure chemicals and mixtures.

II-283

Potency ranking of dermal sensitizing chemicals using the IVSA and epiCS® skin tissues

George DeGeorge[1], Lisa Pratt[1], Matthew Troese[1], Dirk Weisensee[2] and Oliver Engelking[2]

[1]MB Research Labs, Spinnerstown, PN, United States;
[2]Cell Systems Biotechnologie GmbH, Troisdorf, Germany
mbweb@mbresearch.com

Reconstructed human epidermis has been shown to release IL-18 in response to dermal sensitizers. The exposure concentrations resulting in a greater than a threshold positive response (SI ≥ 2.0) correlates with their potency in the *In Vitro* Sensitization Assay (IVSA). In our experiments, NBB and DNCB were strong inducers of IL-18 secretion (EC2.0 = 0.028% and 0.03%). Isoeugenol (IE) and Cinnamaldehyde (CA) were moderate sensitizers, while Resorcinol and HCA (EC2.0 = 22%) were weak sensitizers. Sensitizer potency ranked as follows: NBB > DNCB > PPD, IE ≈ CA > RES > HCA, with NBB, DNCB and PPD classified as strong, IE and CA as moderate, and RES and HCA classified as weak sensitizers. Of the total of 20 chemicals tested, 7 were irritants, 2 were non-sensitizers and only Chlorobenzene was incorrectly predicted as a weak sensitizer. In summary, measuring IL-18 release from RHE allows for highly accurate and sensitive identification of dermal sensitizers.

II-307

Testing strategies for UN GHS classification for serious eye damage/eye irritation of chemicals: Cosmetics Europe analysis

Nathalie Alépée[1], Els Adriaens[2], Daniel Bagley[3], Bertrand Desprez[4], Jalila Hibatallah[5], Karsten Mewes[6], Uwe Pfannenbecker[7], Àlvar Sala[8] and Pauline McNamee[9]

[1]L'Oréal Research & Innovation, Aulnay sous Bois, France; [2]Adriaens Consulting, Aalter, Belgium; [3]Colgate-Palmolive, Piscataway, NJ, United States; [4]Cosmetics Europe, Brussels, Belgium; [5]Chanel Parfums Beauté, Neuilly sur Seine, France; [6]Henkel AG & Co. KGaA, Düsseldorf, Germany; [7]Beiersdorf, Hamburg, Germany; [8]Kao Corporation, S.A, Barcelona, Spain; [9]The Procter & Gamble Company, Egham, United Kingdom
nalepee@rd.loreal.com

A core part of the Cosmetics Europe (CE) eye programme focuses on data integration/evaluation of testing strategies/approaches for identification of serious eye damage/eye irritation of chemicals that can be advocated for external/regulatory acceptance. To enable this, CE curated an initial database of chemicals for which *in vivo* and partial *in vitro* data exist. This database was used for selection of 80 chemicals tested in *in vitro* test methods in the CEFIC CON4EI project. After integration of all *in vitro* data on an industry platform level, remaining data gaps were identified. CE completed *in vitro* testing to fill these data gaps resulting in a comprehensive *in vivo*/*in vitro* database of more than 110 chemicals to date. Building on proposed CON4EI testing strategies, CE has analysed the comprehensive database to determine the robustness of such testing strategies and to identify where opportunities exist for refinement. The outcome of this analysis is presented in the poster.

II-335

Quality considerations: Redefining test systems from animals to tissues and beyond

Amanda Ulrey, Erin Hill and Hans Raabe

Institute for In Vitro Sciences, Inc., Gaithersburg, MD, United States
aulrey@iivs.org

The use of non-whole animal test methods transforms the way regulatory requirements are applied in preclinical testing. Recent global regulatory initiatives emphasize the importance of transitioning to human relevant assays and test systems that do not use animals. When these methods are moved from research into the regulated arena, GLP principles must be followed. The GLPs were originally written in the 1970s, when the vast majority of regulated research was performed using animals as the test system. Current innovative, alternative test systems include *ex vivo* tissues, manufactured biological systems, three-dimensional tissue constructs, and cell cultures maintained in dynamic flow bioreactors. Each type of alternative test system raises new quality and compliance points to consider when used within a regulatory context. Just as the applications of these methods have advanced with regulatory acceptance, the quality control and compliance of these test systems must also progress.

II-343

In vitro co-culture assay for identification of dermal sensitizers

George DeGeorge, Matthew Troese and Lisa Pratt

MB Research Labs, Spinnerstown, PN, United States
mbweb@mbresearch.com

We co-cultured a reconstructed human epidermal tissue (*RHE*) with human plasmacytoid Dendritic Cells (pDCs) for use as a dermal sensitization assay. *RHE* tissues were placed at the air-liquid interface above a media suspension of pDC. The tissues are topically dosed with test materials. After 4 hours of incubation, the *RHE* tissues and pDC were separately cultured for an additional 20 hours. Media was analyzed for IL-18 by ELISA, and pDC were analyzed for changes in CD86 expression. A positive response from the RHE tissues was defined as a 2-fold increase in IL-18 secretion, and for pDCs, a 1.5-fold increase in CD86 expression. Increases in both secretion of IL-18 and expression of CD86 were detected after exposure to dermal sensitizers. A prediction model was developed where a chemical is defined as a sensitizer if either a positive result occurs in either the IL-18 or in CD86. From three individual experiments, we obtained an accuracy of 100%, 83%, and 83%.

II-344

Resolving severe from corrosive irritant ocular classifications using an alternative dual *ex vivo* assay system

George DeGeorge, Puneet Vij, Micheal Carathers, Blair Yasso and Bennett Varsho

MB Research Labs, Spinnerstown, PN, United States
mbweb@mbresearch.com

The classification of severe ocular irritation as distinct from corrosion is determined by assessing reversibility of damage to the corneal epithelium. Here, we demonstrate a dual *ex vivo* assay (Bovine Corneal Opacity and Permeability (BCOP) and Porcine Corneal Opacity Reversibility Assay (PorCORA)) for distinguishing moderately/severely irritating from corrosive substances. Chemicals with known EPA classifications were first evaluated using BCOP assay. Test substances with an *In Vitro* Irritation Score (IVIS) ≥ 20 were considered moderately irritating to corrosive and were further assayed in the PorCORA. In PorCORA, substances causing stain retention persisting passed Day 21 were deemed corrosive and if cleared by Day 21, classified as severe irritant. Of the 21 chemicals, 6 of 6 Category I chemicals induced irreversible damage, while ocular damage caused by 15 of 15 Category II and III chemicals completely reversed by Day 21 in PorCORA.

II-345

Further evaluation of chemicals and mixtures for skin sensitization potential and potency using a reconstructed human epithelium tissue model and the IVSA

George DeGeorge[1], Matthew Troese[1], Bennett Varsho[1] and Dirk Weisensee[2]

[1]MB Research Labs, Spinnerstown, PN, United States; [2]Cell Systems Biotechnologie GmbH, Troisdorf, Germany
mbweb@mbresearch.com

Skin releases IL-18 in response to dermal sensitizers. Using a 3D skin model (epiCS®) in the *In Vitro* Sensitization Assay (IVSA), we measured IL-18 secretion as a biomarker of sensitization. We are able to achieve 90% accuracy when testing 20 chemicals. Analysis of the data revealed that test chemical concentrations that induced a 2-fold increase in IL-18 secretion (Stimulation Index; SI-2) was proportional to the potency of the sensitizer. In the epiCS® IVSA test system tissues can be topically dosed, like skin, with a wide variety of substances, so we obtained sensitizing and non-sensitizing mixtures from commercial sources; including hair dyes, caulking, adhesives, antimicrobial fuel additives and propolis (dietary supplement). A positive response (SI ≥ 2) was detected for all the known sensitizing mixtures. In summary, the IVSA was able to correctly distinguish pure sensitizing chemicals and mixtures from non-sensitizing materials with high accuracy and sensitivity.

II-349

Potency classifications for contact dermal sensitization as determined by the h-CLAT assay

Bennett Varsho, Micheal Carathers and George DeGeorge

MB Research Labs, Spinnerstown, PN, United States
bvarsho@mbresearch.com

We have performed validation studies using the Human Cell Line Activation Test (h-CLAT) and analyzed THP-1 cell responses, alone and in co-culture with human plasmacytoid dendritic cells, to a recommended set of validation chemicals. Test set chemicals include DNCB, Isoeugenol (IE), Cinnamaldehyde (CA), and the non-sensitizing irritants Lactic Acid (LA) and Salicylic Acid (SA). All chemicals were able to be exposed at a low or non-irritating concentration, yielding a CV75 or higher viability. Sensitizer potency was measured by the concentration of test chemical that induced a Stimulation Index (SI) that was a threshold positive response (CD86 = 2.0, CD54 = 1.5) on either cell type. DNCB yielded well above the SI cutoffs with increases at 0.0004%. The potency ranking of the chemical test set we analyzed was DNCB > IE ≈ CA > LA = SA, which are correctly ordered as per human and LLNA potency class (strong, moderate, weak sensitizer, non-sensitizer).

II-416

The importance of understanding physico-chemical properties of chemicals in the evaluation of serious eye damage/eye irritation: Cosmetic Europe analysis

Bertrand Desprez[1], Els Adriaens[2], Nathalie Alépée[3], Dan Bagley[4], Jalila Hibatallah[5], Karsten R. Mewes[6], Uwe Pfannenbecker[7], Àlvar Sala[8] and Pauline McNamee[9]

[1]Cosmetics Europe, Brussels, Belgium; [2]Adriaens Consulting BVBA, Aalter, Belgium; [3]L'Oréal Research & Innovation, Aulnay sous Bois, France; [4]Colgate-Palmolive, Piscataway, NJ, United States; [5]Chanel Parfums Beauté, Neuilly sur Seine, France; [6]Henkel AG & Co KGaA, Düsseldorf, Germany; [7]Beiersdorf AG, Hamburg, Germany; [8]Kao Corporation, S.A, Barcelona, Spain; [9]The Procter & Gamble Company, Egham, United Kingdom
bdesprez@cosmeticseurope.eu

An important part of Cosmetics Europe (CE) eye programme is understanding physico-chemical properties of chemicals and integrating these with *in vitro* test methods to refine/improve performance of testing strategies/approaches to identify serious eye damage/eye irritation. To address this, an exploratory analysis was performed to investigate the relationship between physico-chemical properties (LogP, melting point, vapor pressure, water solubility, surface tension, number of H bond donors/acceptors) and UN GHS classification of chemicals by using principal components analysis (PCA). PCA was performed on different subgroups of chemicals selected from the CE Draize eye Reference Database. Based on the first two components, it was possible to discriminate between chemicals requiring and those not requiring classification for serious eye damage/eye irritation in the datasets. Furthermore, the importance of the parameters and discriminative ability differed between subgroups of chemicals.

II-425

Applicability domain characterization of the SkinEthic HCE reconstructed human corneal test method and performance signature by ingredient classes for serious eye damage/eye irritation evaluation

Nathalie Alépée, Virginie LeBlanc, Marie Helene Grandidier, Laurent Nardelli and Jose Cotovio

L'Oréal, R&I, Aulnay sous Bois, France

nalepee@rd.loreal.com

To demonstrate the large applicability domain of the validated test method SkinEthic™ Human Corneal Epithelium (HCE) Eye Irritation Test (EIT) for chemical categorization, 105 liquids and 95 solids among which mono or multi-constituent substances (including polymers) were tested. They are assigned into 3 classes: organics, inorganics and surfactants.

The predictive capacity was explored for each ingredient classes and showed sensibility systematically greater than 95%, without any Cat 1 chemical misclassified as No Cat. The specificity was at least 87% except for organics ingredients (69%). Accuracy was always greater than 82%, without any misclassification attributed to specific *in vivo* drivers or chemical groups. SkinEthic™ HCE test method is thus applicable to large ingredients categories without any *in vivo* drivers and/or chemical class restrictions, allowing its inclusion in OECD Test Guideline and IATA.

II-438

A novel *in vitro* assay for sensitizers in a purely aqueous system: The modified IL-8 Luc assay using X-VIVO™ 15 as a solvent

Setsuya Aiba[1], Yutaka Kimura[2] and Chizu Fujimura[3]

[1]Department of Dermatology, Tohoku University Graduate School of Medicine, Jordan; [2]Department of Dermatology, Tohoku University Hospital, Jordan; [3]Tohoku University Hospital, Jordan

saiba@med.tohoku.ac.jp

In all current *in vitro* methods to detect sensitizers, DMSO is used to dissolve water-insoluble chemicals. Recently, we demonstrated that a synthetic culture medium, X-VIVO™ 15, can replace DMSO in dissolving water-insoluble chemicals in the IL-8 Luc assay and that the modified IL-8 Luc assay using X-VIVO™ 15(mIL-8 Luc) was significantly better in detecting sensitizers than the original assay (oIL-8 Luc). In this study, we first increased the number of chemicals examined to confirm the mIL-8 Luc's superior performance. Next, we next examined the correlation between the mIL-8 Luc minimum induction concentrations (MITs) and the oIL-8 Luc MITs. We found a significant correlation between the MITs of the mIL-8 Luc and oIL-8 Luc (R2 = 0.866). We then examined the correlation between mIL-8 Luc MITs and parameters of other *in vitro* test methods, i.e., DPRA, h-CLAT, and KeratinoSens. Only weak correlation was observed between mIL-8 Luc MITs and CD86 EC150 and CD54 EC200. These results demonstrate that the mIL-8 Luc is a unique *in vitro* test for detecting sensitisers in a purely aqueous phase and that the obtained data cannot be replaced by the data from any other test methods.

Reference

Kimura, Y., Fujimura, C., Ito, Y. et al. (2015). Optimization of the IL-8 Luc assay as an in vitro test for skin sensitization. *Toxicol In Vitro 29*, 1816-1830.

II-451

Investigation of nephrotoxicity and its mode of action by metabolomics *in vitro*

Barbara Birk[1], Saskia Sperber[1], Andreas Verlohner[1], Hans-Albrecht Huener[1], Tilmann Walk[2], Volker Haake[2], Tzutzuy Ramirez Hernandez[1], Hennicke Kamp[1] and Bennard van Ravenzwaay[1]

[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany; [2]Metanomics GmbH, Berlin, Germany

barbara.birk@basf.com

The development and application of alternatives to animal testing has considerably increased in the last years but relevant toxicological endpoints (systemic and reproductive toxicity) can currently not fully be replaced by *in vitro* studies and the mode of action of toxic compounds is still unclear. BASF has established successfully Metabolomics *in vitro* in the liver. Now, metabolomics *in vitro* in kidney cells is a novel approach that can enable the identification of nephrotoxicity including mode of action. We report on the establishment of a NRK-52e-based cell system. Details about the cultivation, treatment and sensitive harvesting of the cells, the metabolome analysis (> 200 metabolites) and the reproducibility of the data will be presented. These results strongly suggest that this technology is now ready for testing substances with different mode of action to validate this method. Metabolomics *in vitro* in kidney cells might be a new animal-free method for investigation of nephrotoxicity, an important part of systemic toxicity.

II-468

Lessons from read-across case studies for repeated-dose toxicity

Catherine Mahony[1], Terry Schultz[2] and Mark Cronin[3]

[1]Procter & Gamble, Guildford, United Kingdom; [2]College of Veterinary Medicine, The University of Tennessee, TN, United States; [3]School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool, United Kingdom

mahony.c@pg.com

A series of case studies designed to further acceptance of read-across (RA) predictions have been published with the aim of determining sources of uncertainty associated with RA. While there is uncertainty associated with the presumptions of RA from source- to target-chemicals, the justification hinges on 1) the quality and quantity of the RA data, and 2) the similarity justification. We have learned that uncertainties associated with RA are typically brought about by deficiencies in underlying knowledge and data. Similarity in chemistry is not enough to justify a RA prediction; rather toxicokinetic and toxicodynamic similarity is essential. Non-animal methods often provide critical information needed to strengthen the toxicodynamic similarity rationale. Toxicokinetic data, especially metabolism, is often lacking.

II-477

Genetic variability in a frozen batch of MCF-7 cells invisible in routine authentication affecting cell function

Andre Kleensang, Marguerite M. Vantangoli, Shelly Odwin-DaCosta, Melvin E. Andersen, Kim Boekelheide, Mounir Bouhifd, Albert J. Fornace Jr, Heng-Hong Li, Carolina B. Livi, Samantha Madnick, Alexandra Maertens, Michael Rosenberg, James D. Yager, Liang Zhao and Thomas Hartung

Johns Hopkins University, Baltimore, MD, United States
akleens1@jhu.edu

Common recommendations for cell line quality control fall short addressing genetic heterogeneity. We demonstrate that there can be marked cellular and phenotypic heterogeneity in a single batch of the human breast adenocarcinoma cell line MCF-7 obtained directly from ATCC that are invisible with the usual cell authentication by STR markers. STR profiling just fulfills the purpose of authentication testing, which is to detect significant cross-contamination and cell line misidentification. This heterogeneity can have serious consequences for reproducibility of experiments as shown by morphology, estrogenic growth dose-response, whole genome gene expression and untargeted mass-spectroscopy metabolomics. Using Comparative Genomic Hybridization (CGH), differences were traced back to genetic heterogeneity present already in the cells from the original frozen vials from the same ATCC lot, however, STR markers did not differ from ATCC reference for any sample. These findings underscore the need for additional quality assurance in Good Cell Culture Practice to reveal possible genomic heterogeneity and genetic drifts within cell lines.

Reference

Kleensang, A., Vantangoli, M. M. and Odwin-DaCosta, S. (2016). Genetic variability in a frozen batch of MCF-7 cells invisible in routine authentication affecting cell function. *Sci Rep 6,* 28994. doi:10.1038/srep28994

II-505

The borderline range of prediction models for skin sensitisation potential assessment: Quantification and implications for evaluating non-animal testing methods precision

Maria Leontaridou[1], Daniel Urbisch[2], Susanne Kolle[2], Katharina Ott[2], Dennis Mulliner[3], Silke Gabbert[1] and Robert Landsiedel[2]

[1]Environmental Economics and Natural Resources Group, Wageningen University, Wageningen, The Netherlands; [2]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany; [3]Computational Chemistry and Biology, BASF SE, Ludwigshafen, Germany
maria.leontaridou@wur.nl

Testing methods to assess the skin sensitisation potential of a substance usually use threshold criteria to dichotomise continuous experimental read-outs into yes/no conclusions. The threshold criteria are prescribed in the respective OECD test guidelines and the conclusion is used for regulatory hazard assessment, i.e. classification and labelling of the substance. Due to biological and technical variability, we can identify a borderline range (BR) around the classification threshold within which test results are non-conclusive.

We quantify BRs in the prediction models of the non-animal testing methods DPRA, LuSens and h-CLAT, and of the animal test LLNA, respectively. Depending on the size of the BR we find that between 6% and 28% of the substances were considered borderline. Based on our findings we propose expanding the standard binary classification of substances into 'positive'/'negative' by adding a 'non-conclusive' alert for cases where test results fall within the borderline range.

Reference

Leontaridou, M., Urbisch, D., Kolle, S. N. et al. (2017). Quantification of the borderline range and implications for evaluating non-animal testing methods' precision. *ALTEX*, Epub ahead of print. doi: 10.14573/altex.1606271

II-516

Cosmetics Europe eye programme: Relevance to integrated approaches on testing and assessment for eye hazard

Pauline McNamee[1], Nathalie Alépée[2], Els Adriaens[3], Daniel Bagley[4], Bertrand Desprez[5], Jalila Hibatallah[6], Karsten Mewes[7], Uwe Pfannenbecker[8] and Àlvar Sala[9]

[1]The Procter & Gamble Company, Egham, United Kingdom; [2]L'Oréal Research & Innovation, Aulnay sous Bois, France; [3]Adriaens Consulting, Aalter, Belgium; [4]Colgate-Palmolive, Piscataway, NJ, United States; [5]Cosmetics Europe, Brussels, Belgium; [6]Chanel Parfums Beauté, Neuilly sur Seine, France; [7]Henkel AG & Co. KGaA, Düsseldorf, Germany; [8]Beiersdorf AG, Hamburg, Germany; [9]Kao Corporation, S.A, Barcelona, Spain
mcnamee.pm@pg.com

Overall aim of Cosmetics Europe (CE) eye program is to advocate towards better recognition by regulators/external scientific organizations of safety assessment approaches using testing strategies based on alternative methods. The CE program comprises three core elements: 1) method evaluation through optimization/refinement of existing *in vitro* test methods; 2) guidance for industry on selection of chemicals for use in development/evaluation of alternative methods/testing strategies through provision of a comprehensive database of existing *in vivo* data analysed by drivers of classification and 3) data integration/evaluation of testing strategies/approaches. The outcome of each project provides a means to inform different elements of the modules within the OECD guidance on integrated approaches on testing and assessment (IATA). This presentation describes how each project of the eye program contributes to the different modules across the three parts of the IATA.

II-558

CON4EI: EpiOcular eye irritation tests – OECD TG 492 and ET-50 (time-to-toxicity) protocols

Silvia Letasiova[1], Helena Kandarova[1], Els Adriaens[2], Agnieszka Drzewiecka[3], Przemyslaw M. Fochtman[3], Katarzyna D. Gruszka[3], Robert Guest[4], Gareth Maglennon[4], Jane Schofield[5], Jamin A. Willoughby Sr[6], Nathalie Alépée[7], Sandra Verstraelen[8] and An R. Van Rompay[8]

[1]MatTek *In Vitro* Life Science Laboratories, Bratislava, Slovakia; [2]Adriaens Consulting BVBA, Aalter, Belgium; [3]Institute of Industrial Organic Chemistry Branch Pszczyna, Pszczyna, Poland; [4]ENVIGO, Shardlow, Derby, United Kingdom; [5]ENVIGO, Cambridgeshire, United Kingdom; [6]Cyprotex US, Kalamazoo, MA, United States; [7]L'Oréal Research & Innovation, Aulnay sous Bois, France; [8]VITO NV, Applied Bio & Molecular Systems, Mol, Belgium

sletasiova@mattek.com

Assessment of the acute eye irritation potential is a part of the international regulatory requirements for testing of chemicals. The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation Testing Strategy) project is to develop tiered testing strategies for eye irritation assessment for all drivers of classification. For this, a set of 80 reference chemicals was tested with eight different alternative methods. Here, the results obtained with reconstructed human cornea-like epithelium EpiOcular and the two Eye Irritation Tests are shown.

The primary aim was to evaluate the ability of the test methods to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling. In addition, the predictive capacity in terms of *in vivo* driver of classification was investigated. In a second step, it was investigated whether the EpiOcular EIT and ET-50 can be used also for prediction of subclasses of ocular irritation.

For the EpiOcular EIT (OECD TG 492), a sensitivity of 96.9% and specificity of 86.7% with an accuracy of 95% was obtained (100% concordance). For the EpiOcular ET-50 method (time-to-toxicity test), the overall accuracy of 74.5%, an False Negative Rate of 3.1% and False Positive Rate of 3.4% were achieved. Furthermore, about 79% of the Cat 1 liquids and 69% of the Cat 1 solids and 68% of the Cat 2 liquids and about 61% of the Cat 2 solids were identified correctly in the time-to-toxicity test. The results of these studies seem promising with regard to the evaluation of inclusion of EpiOcular test methods into an integrated testing strategy (ITS) for eye irritation assessment.

II-568

CON4EI: Short Time Exposure (STE) test method for hazard identification and labelling of eye irritating chemicals

Els Adriaens[1], Jamin Willoughby[2], Nathalie Alépée[3], Helena Kandarova[4], Przemyslaw Fochtman[5], Robert Guest[6], Sandra Verstraelen[7] and An Van Rompay[8]

[1]Adriaens Consulting bvba, Aalter, Belgium; [2]Cyprotex US, LLC, Kalamazoo, MI, United States; [3]L'Oréal, Research & Innovation, Aulnay sous Bois, France; [4]MatTek, Bratislava, Slovakia; [5]Institute of Industrial Organic Chemistry, Pszczyna, Poland; [6]Envigo, Cambridge, United Kingdom; [7]Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium; [8]Vito, Antwerp, Belgium

adriaens.consulting@telenet.be

The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation) project was to develop tiered testing strategies for eye irritation assessment that can lead to complete replacement of the *in vivo* Draize rabbit eye test (OECD TG 405). A set of 80 reference chemicals was tested with seven test methods, one method was the Short Time Exposure (STE, OECD TG 491) assay that measures the viability of SIRC rabbit corneal cells after 5 min exposure to 5% and 0.05% of test material. The method can be used to identify Cat 1 and No Cat chemicals. The accuracy of the STE test method to identify Cat 1 chemicals was 61.3% with 23.7% sensitivity and 95.2% specificity. Excluding non-soluble chemicals and unqualified results, had no effect on the performance to identify Cat 1 chemicals (accuracy 62.2% with 22.7% sensitivity and 100% specificity). The accuracy of the STE test method to identify No Cat chemicals was 72.5% with 66.2% sensitivity and 100% specificity. Excluding highly volatile chemicals, non-surfactant solids and non-qualified results resulted in an important improvement of the performance of the STE test method (accuracy 96.2%, 81.8% sensitivity and 100% specificity).

II-571

CON4EI: Selection of the reference chemicals for hazard identification and labelling of eye irritating chemicals

Els Adriaens[1], Nathalie Alépée[2], Helena Kandarova[3], Przemyslaw Fochtman[4], Robert Guest[5], Jamin Willoughby[6], Sandra Verstraelen[7] and An Van Rompay[8]

[1]Adriaens Consulting bvba, Aalter, Belgium; [2]L'Oréal, Research & Innovation, Aulnay sous Bois, France; [3]MatTek, Bratislava, Slovakia; [4]Institute of Industrial Organic Chemistry, Pszczyna, Poland; [5]Envigo, Cambridge, United Kingdom; [6]Cyprotex US, LLC, Kalamazoo, MI, United States; [7]Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium; [8]Vito, Antwerp, Belgium

adriaens.consulting@telenet.be

In the past, several prospective and retrospective validation studies have taken place in the area of serious eye damage/eye irritation testing. Success in terms of complete replacement of the regulatory *in vivo* Draize rabbit eye test has not yet been achieved. A very important aspect to ensure development of successful alternative test methods and/or strategies for serious eye damage/eye irritation testing is the selection of appropriate reference chemicals. A set of 80 reference chemicals was selected for the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project, in collaboration with Cosmetics Europe (CE), from the Draize Reference Database published by CE based on key criteria that were set in their paper (e.g. balanced by important driver of classification and physical state). The set of chemicals was tested in seven alternative test methods. Detailed background on selection of the test chemicals is provided.

II-578

CON4EI: Slug Mucosal Irritation (SMI) test method for hazard identification and labelling of eye irritating chemicals

Els Adriaens[1], Nathalie Alépée[2], Helena Kandarova[3], Przemyslaw Fochtman[4], Robert Guest[5], Jamin Willoughby[6], Sandra Verstraelen[7] and An Van Rompay[8]

[1]Adriaens Consulting bvba, Aalter, Belgium; [2]L'Oréal, Research & Innovation, Aulnay sous Bois, France; [3]MatTek, Bratislava, Slovakia; [4]Institute of Industrial Organic Chemistry, Pszczyna, Poland; [5]Envigo, Cambridge, United Kingdom; [6]Cyprotex US, LLC, Kalamazoo, MI, United States; [7]Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium; [8]Vito, Antwerp, Belgium

adriaens.consulting@telenet.be

The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project was to develop tiered testing strategies for eye irritation assessment that can lead to complete replacement of the *in vivo* Draize rabbit eye test (OECD TG 405). A set of 80 reference chemicals was tested with seven test methods, one method was the Slug Mucosal Irritation (SMI) test method. The method measures the amount of mucus produced (MP) during a 1-hour contact with a 1% and 10% dilution of the chemical. Based on the MP, a classification (Cat 1, Cat 2, or No Cat) is predicted. The performance of the SMI test method to identify Cat 1 vs Not Cat 1 chemicals was 78.6% correct with 65.8% sensitivity and 90.5% specificity. The accuracy of the SMI test method to identify No Cat vs Classified (Cat1/Cat 2) chemicals was 78.6% with 76.9% sensitivity and 86.7% specificity. The SMI test method may be successful when used as a first or second step to identify Cat 1 in a two- or three-step strategy.

II-583

Tracking the successful implementation of Tox21 principles

Vicki Katrinak[1] and Catherine Willett[2]

[1]Animal Research Issues, The Humane Society of the United States, Gaithersburg, MD, United States; [2]Regulatory Toxicology, Risk Assessment and Alternatives, The Humane Society of the United States, Gaithersburg, MD, United States

vkatrinak@humanesociety.org

Ten years ago, the National Academy of Sciences released its groundbreaking report, *Toxicity Testing in the 21st Century, A Vision and a Strategy*. This seminal report highlighted the importance of moving away from traditional animal tests and toward new non-animal approaches for assessing the safety of chemicals. These new testing strategies can provide data more quickly and at a significant cost-savings, utilize the latest scientific advances, eliminate concerns with extrapolating animal data to people, and advance animal welfare. By reviewing the abstracts and posters presented at the Society of Toxicology Annual Meetings, it is evident that there has been a fundamental shift in how scientists and regulators view chemical safety substantiation. This presentation will highlight the changing landscape for toxicity testing as toxicologists have developed and implemented 21st century science over the past ten years.

II-628

The Brazilian challenge: Adopting 17 alternative methods in five years

Antoniana Ottoni

Research and Toxicology, Humane Society International, Brasília, Brazil
aottoni@hsi.org

In 2014, the National Council for the Control of Animal Experimentation (CONCEA) recognized 17 alternative testing methods to be implemented within five years in Brazil. This framework, which is about to become reality, will impact several sectors, as it includes the most common toxicological endpoints, such as: skin irritation and corrosion (OECD TG 430, 431, 435 and 439), eye irritation and corrosion (OECD TG 437, 438 and 460), phototoxicity (OECD TG 432), skin absorption (OECD TG 428), skin sensitization (OECD TG 429, 442A and 442B), acute toxicity (OECD TG 420, 423, 425 and 129) and genotoxicity (OECD TG 487). In this scenario, the present work aims to show what the Brazilian authorities are doing to guarantee the infrastructure capacity for the implementation of these methods. It also discusses how Brazilian authorities are updating their regulations, taking into account the deadline of June 2019.

References

Andersen, M. E. and Krewski, D. (2009). Toxicity testing in the 21st century: Bringing the vision to life. *Toxicol Sci 107*, 324-330.

Brasil. Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada – RDC n°35 07/08/2015.

Brasil. Lei n° 7.802 de 11 de julho de 1989.

Hartung, T. (2009). Toxicology for the twenty-first century. *Nature 460*, 208-212.

Hartung, T. and Busquet, F. (2017). The need for strategic development of safety science. *ALTEX 34*, 3-21.

Tréz, T. (2015). Experimentação animal: Um obstáculo ao avanço científico. Porto Alegre: Tomo Editorial, 264p.

II-631

Developmental toxicity potency of valproate analogues in a human pluripotent stem cell-based assay

Jessica Palmer[1], Alan Smith[1], Robert Burrier[1], Elizabeth Donley[1], Fred Kirchner[1], Dinant Kroese[2], Regina Stöber[3] and Nicole Kleinstreuer[4]

[1]Stemina Biomarker Discovery, Madison, WI, United States; [2]The Netherlands Organization for Applied Scientific Research (TNO), The Hague, The Netherlands; [3]IfADo – Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany; [4]National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC, United States
jpalmer@stemina.com

The development and use of alternative models for safety screening in place of animal models has been at the forefront of the toxicology field for over a decade; however, application of these assays in a regulatory setting is still poorly understood. The EU-ToxRisk project has developed several case studies to address this issue. One of these case studies investigates the teratogenic potency of several valproate (VPA) analogues. The devTOX *quick*Predict platform is an *in vitro* human pluripotent stem (hPS) cell-based assay that predicts the developmental toxicity potential of chemicals based on changes in hPS cell metabolism. The assay has been used by multiple industries and, of note, by the United States Environmental Protection Agency (EPA) and National Toxicology Program (NTP) in support of Tox21. In this study, we tested ten VPA analogues included in the EU-ToxRisk case study with the devTOX *quick*Predict platform and ranked their developmental toxicity potential. Historical data had shown that the assay is highly concordant (~85%) with human and *in vivo* developmental toxicity outcomes across a diverse set of chemotypes.

II-687

Silver nanoparticles dosimetry-response comparison in *in vitro* primary organotypic C57BL/6 and A/J mouse midbrain micromass cultures at various developmental stages

Julie Juyoung Park[1,2], Brittany A. Weldon[1,2], Sungwoo Hong[1], Tomomi Workman[2], Russell Dills[1], William C. Griffith[2], Terrance J. Kavanagh[1] and Elaine M. Faustman[1,2]

[1]Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, WA, United States; [2]Institute of Risk Analysis and Risk Communication, Seattle, WA, United States
julie814@uw.edu

Silver nanoparticle (AgNP) exposures during neurodevelopment were assessed using a modified *in vitro* 3D mouse micromass (MM) system. AgNPs are widely used in consumer products and medical devices for their antimicrobial properties, but have been challenging to evaluate in *in vitro* models. We found that the effects of AgNPs on neuronal growth, proliferation, and differentiation in both C57BL/6 and A/J mice derived MM cells to be similar. MM cells were not affected by particle coatings, but were more sensitive to 20 nm sized AgNP at all developmental times. Of three times evaluated *in vitro* (DIV 8, 15, and 22) with dosimetry, C57BL/6 were most susceptible at DIV 8 while at DIV 15 for A/J. Yet, both time points were associated with similar impacts on early differentiation, suggesting that this period is a "window of susceptibility" for AgNPs. These models offer approaches to assess both environmental and genetic factors affecting neurodevelopmental susceptibility when exposure is evaluated using dosimetry.

Theme III – Innovative Models for Safety and Efficacy

Coordinators
Ellen Frische, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
Ivan Rusyn, Texas A&M University, College Station, TX, United States
Jeetu Eswaraka, Amgen Inc., Thousand Oaks, CA, United States
Maurice Whelan, EURL ECVAM, JRC, Ispra, Italy

Session III-1: Oral Presentations

Session III-1: Models of the Respiratory System

Co-Chairs
William Altemeier, University of Washington, Seattle, WA, United States
Cindy Pekow, Veteran's Administration, Dept. of Comparative Medicine, Seattle, WA, United States

III-1-837

Refinement, reduction, and replacement of animal models in respiratory research

Cindy A. Pekow
Veteran's Administration, Department of Comparative Medicine, University of Washington, Seattle, WA, United States

Animal models have been key to understanding respiratory function and disease states, aiding in development of preventive modalities, treatments, and cures. This session will explore refinements that improve the welfare of animal models that continue to be vital to this research. Refinements include developing humane endpoints, including use of scoring rubrics that incorporate specific parameters related to the model in use, such as respiratory distress, or degree of hypoxia. Use of analgesics, and non-pharmaceutical comfort methods may improve animal welfare as well. Technological advances have moved investigations to the cellular and molecular level, replacing live animals in many respiratory research studies. Additional discussion will highlight technology and methods that reduce the number of research animals and replace the need for live animals in this work.

III-1-836

Development of human organotypic culture systems to model airway biology

William Altemeier[1], James D. Nolin[1], Ying Lai[1], Michael S. Mulligan[2] and Teal S. Hallstrand[1]
[1]Center for Lung Biology and Department of Medicine, University of Washington, Seattle, WA, United States; [2]Center for Lung Biology and Department of Surgery, University of Washington, Seattle, WA, United States

Organotypic culture systems are a valuable tool for studying cellular interactions, *in vitro*, and modeling clinically relevant diseases or exposures. For the respiratory system, airway epithelial cells differentiated at an air-liquid phenotype develop a stratified epithelial layer composed of ciliated cells, mucus producing cells, and Club cells. We have developed a co-culture system, using primary airway epithelial cells isolated from cadaveric bronchial rings and either mast cells derived from CD34-selected cord blood cells or eosinophils isolated by negative selection from peripheral blood. Using this system, we have demonstrated that a feed-forward system between epithelial cells and mast cells augments epithelial cell IL33 production.

III-1-347

A co-culture of M1 and M2 type macrophages with primary bronchial epithelial cells cultured at the air-liquid interface (ALI-PBEC)

Cyrille Krul[1], Padmini Khedoe[2], Marloes Hofstee[2], Annemiek Schouten[2], Ingeborg Kooter[3] and Pieter Hiemstra[2]
[1]University of Applied Sciences Utrecht, Utrecht, The Netherlands; [2]Pulmonology, Leiden University Medical Center, Leiden, The Netherlands; [3]TNO, Zeist, The Netherlands
cyrille.krul@hu.nl

Current cell culture lung models are mostly based on the use of a single cell type, such as airway epithelial cells (AEC). However, AEC are in close contact with immune, inflammatory and other structural cells in the lung and play a central role in chronic obstructive pulmonary diseases. Therefore, current models should be extended to better mimic the airway mucosa. We focused on the interaction between human macrophages and AEC using a state-of-the-art 3D model of human primary bronchial epithelial cells cultured at the air-liquid interface (ALI-PBEC) to obtain fully differentiated epithelial cells. Human $CD14^+$ monocytes were differentiated towards either a pro-inflammatory (M1) or anti-inflammatory, pro-repair (M2) phenotype. Interestingly, LPS-activated M1 type (pro-inflammatory) macrophages induce expression of hBD2, IL-8 and IL-6 in ALI-PBEC at 24 h, whereas this response was lower upon co-culture of ALI-PBEC with LPS-stimulated M2 after 24 h. We conclude that especially M1 type macrophages enhance these innate immune responses in ALI-PBEC.

This study was supported by grants from Galapagos, ZonMw (grant # 11401095003) a*nd Stichting Proefdiervrij*.

III-1-84

In vitro phenotypic profiling of human lung cells reveals DNA damage responses commonly induced by pulmonotoxicity chemicals

Jia-Ying Joey Lee, James A Miller, Ting-Zhen Vanessa Kee and Lit-Hsin Loo

Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR), Singapore

leejy@bii.a-star.edu.sg

Bronchial and alveolar epithelial cells (BEC and AEC) are major sites of xenobiotic metabolism, and thus susceptible to xenobiotic-induced toxicity. Although many chemicals are known to cause lung diseases in human, their modes of action are unclear. Here, we report a study to identify predictive *in vitro* endpoints for pulmonotoxicity in human BEC and AEC lines using high-content imaging and the "High-throughput *In-vitro* Phenotypic Profiling for Toxicity Prediction" (HIPPTox) platform (Su et al., 2016). We found several cellular features that indicate DNA damage responses, which can achieve > 80% accuracy in classifying 34 reference chemicals. Furthermore, we confirmed that many of the predicted pulmonotoxic chemicals, including *p*-phenylenediamine (hair dye ingredient), diacetyl (popcorn flavoring) or nitrofurantoin (drug), induce DNA strand breaks under *in vitro* conditions. Our results show that human pulmonotoxicity of these chemicals can be predicted with high efficiency and accuracy.

Reference

Su, R., Xiong, S., Zink, D. and Loo, L.-H. (2016). High-throughput imaging-based nephrotoxicity prediction for xenobiotics with diverse chemical structures. *Arch Toxicol 90*, 2793-2808.

III-1-697

An integrated approach for assessing the inhalation toxicity of nanomaterials

Monita Sharma[1], Barbara Rothen-Rutishauser[2], Hana Barosova[2], Savvina Chortarea[2], Fikad Zerimariam[2], Martin J. D. Clift[3], Vicki Stone[4], Patrick Hayden[5], Anna Maione[5] and Amy J. Clippinger[1]

[1]PETA International Science Consortium Ltd., London, United Kingdom; [2]Adolphe Merkle Institute, University of Fribourg, Switzerland; [3]In Vitro Toxicology Group, Swansea University Medical School, Swansea, Wales, United Kingdom; [4]Heriot-Watt University, Edinburgh, United Kingdom; [5]MatTek Corporation, Ashland, MA, United States

monitas@piscltd.org.uk

Pulmonary fibrosis has been linked to prolonged exposure to multi-walled carbon nanotubes (MWCNTs). Mono- and co-cultures of human cell lines – including alveolar epithelial cells (A549), fibroblasts (MRC-5), and macrophages (THP-1) – were exposed to 2 types of MWCNTs (Mitsui 7s and Nanocyl) at different concentrations in suspension (5, 10, and 20 μg/ml) and at air-liquid interface (ALI) (2-10 μg/cm^2) to assess the pro-fibrotic response. 96 h exposure of mono-cultures to Mitsui-7 induced pro-inflammatory (interleukin (IL)-8, tumor necrosis factor-alpha, and IL-1 beta levels) and pro-fibrotic response (osteopontin levels). Co-cultures exposed at ALI also revealed that longer exposures are more suitable to predict pro-fibrotic effects. This work is complemented by studies in a reconstructed human alveolar tissue model (EpiAlveolar™ , MatTek Corp). When used in an integrated approach with other *in vitro* and *in silico* methods, this system could be used to predict lung toxicity of substances.

III-1-21

Evaluation of an active lung simulator for aerosol inhalation test replacement

Richard Pasteka[1,2] and Mathias Forjan[1,3]

[1]Dept. of Biomedical, Health and Sports Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria; [2]Department of Biomedical Engineering, Brno University of Technology, Brno, Czech Republic; [3]Science and Technology School, Universidade de Tras-os-Montes e Alto Douro, Vila Real, Portugal

richard.pasteka@technikum-wien.at

Aerosols are a ubiquitous part of the surrounding environment. Airborne particles may be produced by intention, as several spray-based cosmetics, but are not intended to be inhaled. The number of deposited particles in the lung tissue can only be estimated using mathematical models or simplified organ models (Park and Wexler, 2008). However, the question of actually deposited particles remains. The presented active lung simulator, i-Lung, has been designed to enable active in- and exhalation and live measurement of environmental aerosols with a particle size distribution of 0.2 μm - 40 μm, representing respirable and inhalable aerosols. The i-Lung simulates an active spontaneously breathing human lung by changing the pressure within the housing of the different applicable lung equivalents as Latex bags or porcine lungs. An extracorporeal perfusion circuit, based on human lung transplantation techniques, is developed alongside and is supposed to nourish porcine lungs, taken from the slaughtering process.

Reference

Park, S. S. and Wexler, A. S. (2008). Size-dependent deposition of particles in the human lung at steady-state breathing. *Aerosol Science 39*, 266-276.

III-1-265

Long term culture of nasal-tracheal-bronchial and bronchiolar human airway epithelia at interconnected and dynamic liquid flow conditions: The SupAir project

Samuel Constant[1], *Mireille Caulfuty*[1], *Gregory Fischler*[2], *Song Huang*[1], *Ludovic Wiszniewski*[1], *Adrien Roux*[2] *and Luc Stoppini*[2]

[1]Epithelix, Plan-les-Ouates, Switzerland; [2]University of Applied Sciences and Arts of Western Switzerland, Geneva, Switzerland
samuel.constant@epithelix.com

We herein report the first interconnection of four fully differentiated epithelia reconstituted from primary human cells from different anatomical origin namely from the nose, the trachea and the bronchi (three versions of the MucilAir™ system) and small airways (SmallAir™).

The system is composed of a culture plate allowing 3D models grown in Transwell to be (i) interconnected *via* the basal compartment through meso-fluidics (0.3 ml/min of a common culture medium) and (ii) maintained at the Air-Liquid Interface.

Stability in term of morphology and function of the four fully differentiated human airway epithelia was evaluated. End points measurement included longitudinal tissue integrity assessment (TEER); Cilia activity (Cilia Beating Frequency) and morphological and histological evaluation (H/E-Alcian blue staining).

The study concluded that minor differences are observed for all tested end-points after 6 weeks of culture at interconnected and dynamic liquid flow conditions, therefore this model allows testing the toxicity of the chemical compounds simultaneously on several anatomical regions of the respiratory tract, as well as the interplay of different organs/tissues *in vitro*.

Session III-2: Models Used in Neurodevelopmental Applications

Co-Chairs
Ellen Fritsche, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
Anna Bal-Price, European Commission Joint Research Centre – Health, Consumers and Reference Materials, Ispra, Italy

III-2-745

Methods for stage-specific developmental neurotoxicity testing with stem-/progenitor cell-based 3D models

Ellen Fritsche

IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
ellen.fritsche@uni-duesseldorf.de

It is now agreed that an *in vitro* testing battery is needed to cover different endpoints at different developmental stages for developmental neurotoxicity (DNT) testing. We have established two assays covering parts of embryonic and fetal development by using hiPSC- and primary human neurospheres, respectively. Differentiation of hiPSC-derived neurospheres thereby mimics early embryonic neuronal migration and differentiation. Development of primary human neurospheres, which are derived from fetal brains, represent their fetal period of origin. The latter are capable of proliferating in 3D in culture and radially migrate and differentiate into neurons and glia cells, i.e. radial glia, astrocytes and oligodendrocytes, thus mimicking basic processes of cortical development. Here, data on assay development, scientific validation and compound testing will be presented that will contribute to defining the biological application domains of the assays. More assays are needed to cover most of the basic neurodevelopmental processes with an *in vitro* testing strategy.

III-2-780

Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA) for identification of potential developmental neurotoxicants

Anna Bal-Price

European Commission Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Ispra, Italy
anna.price@ec.europa.eu

Recent societal concerns have been raised linking the rise in children's developmental learning disabilities to chemical exposure. However, there is a lack of information concerning the developmental neurotoxicity (DNT) hazard posed by industrial and environmental chemicals due to complex, current testing that is entirely based on animal studies. A new testing approach, based on a battery of *in vitro* DNT assays anchored to common key events identified in the existing DNT Adverse Outcome Pathways (AOPs) and critical neurodevelopmental processes will be discussed (Bal-Price et al., 2015a). AOP-Informed Integrated Approaches to Testing and Assessment (IATA) will be proposed for screening and prioritization of chemicals with DNT potential (Bal-Price et al., 2015b). For generation of new data, the IATA framework should be based on a set of non-testing and *in vitro* test methods that can be used in a flexible combination (fit-for-purpose). Such IATA

would facilitate an application of mechanistic knowledge into DNT evaluation, increasing scientific confidence in the decision-making process for regulatory purposes (Bal-Price et al., 2017).

References

Bal-Price, A., Crofton, K. M., Sachana, M. et al. (2015a). Putative adverse outcome pathways relevant to neurotoxicity. *Crit Rev Toxicol 45*, 83-91.

Bal-Price, A., Crofton, K. M., Leist, M. et al. (2015b). International STakeholder NETwork (ISTNET): Creating a developmental neurotoxicity (DNT) testing road map for regulatory purposes. *Arch Toxicol 89*, 269-287.

Bal-Price, A., Lein, P. J., Keil, K. P. et al. (2017). Developing and applying the adverse outcome pathway concept for understanding and predicting neurotoxicity. *Neurotoxicology 59*, 240-255.

III-2-478

3D neural models to study toxicity and disease

Helena Hogberg[1], Celina Abreu Monteiro[2], Paula Barreras[3], Megan Chesnut[1], Lucio Gama[2], Georgina Harris[1], Thomas Hartung[1], Carlos Pardo-Villamizar[3], Lena Smirnova[1], Xiali Zhong[1] and David Pamies[1]

[1]CAAT, Johns Hopkins University, Baltimore, MD, United States; [2]School of Medicine, Johns Hopkins University, Baltimore, MD, United States; [3]Department of Neurology, Johns Hopkins University, Baltimore, MD, United States

hhogber2@jhu.edu

Lately it has become evident that more complex *in vitro* cell models, such as three-dimensional test systems are essential to reproduce the architecture and function of an organ. This holds especially true for the central nervous system (CNS) which consist of numerous different cell types such as, neurons, astrocytes, oligodendrocytes and microglia and the cell-cell interactions are of key importance for brain development and function.

Considering that one astrocyte interacts with numerous synapses, suggests that monolayer cultures are not adequate to capture theses interactions. 2D models restrict the astrocyte shape and prevent the interactions between astrocytes processes and numerous synapses. Moreover, glial cells have an important role in guiding the neurons to the right position in the migration processes during brain development, which is difficult to mimic in monolayer cell cultures.

Moreover, the improved cell-cell interaction in the 3D structure, especially between glial cells and neurons, enhances neurogenesis, synapse formation and axon myelination. In addition to the improved structure and cell connectivity, three-dimensional cultures have shown increased survival and enhanced neuronal differentiation compared to traditional monolayer cultures.

In the recent years, our group has developed several 3D neural models based on rat primary cells, a human dopaminergic cell line, and human induced pluripotent stem cells (iPSCs). The models have been used for numerous different applications, including (developmental) neurotoxicity, Parkinson's disease, cancer, resilience, blood-brain barrier, autism, Down's syndrome, inflammation, Zika, and other virus infections.

The possibility to infect our human iPSCs model with viruses such as Zika and JC-virus is especially exciting as very few animal and *in vitro* cell models been able to recapitulate these pathologies. This indicates that 3D neural models have the potential to replace animal models in toxicology and disease.

Funded by FDA #U01FD004230, NIH #1U18TR000547, ARDF, Colgate-Palmolive.

III-2-673

The controlled formation of perfused vascularized 3D neural constructs and their utilization in neurodevelopmental disease modelling and toxin screening

William Daly[1], Gaurav Kaushik[1], Elizabeth Torr[1], Cheryl Soref[1], Andrew Dias[1], Gianluca Fontana[1], Michael Schwartz[1], Collin Edington[2], Daniel Gil[3], Jessica Antosiewicz-Bourget[3], Melissa Skala[3], Linda Griffith[2], James Thomson[3] and William Murphy[1]

[1]Orthopedics and Rehabilitation, University of Wisconsin Madison, Madison, WI, United States; [2]MIT, Boston, MA, United States; [3]Morgridge Institute, Madison, WI, United States

william.daly@wisc.edu

Modelling the cellular diversity of the developing human brain is a major challenge in neural stem cell engineering and is essential in neural disease modelling and toxin screening. Here, we have generated neural progenitor cells, endothelial cells, and microglial precursors from induced pluripotent stem cells (iPSCs) for inclusion in 3D vascularized cerebral organoids. Synthetic hydrogels formed organoids with high sample uniformity and are a suitable alternative to Matrigel. We have integrated the organoids into two bioreactor systems (a pumped recirculating wellplate and a pumpless microfluidics platform) to perfuse and mature the organoids. Organoids generated in the microfluidics platform were used to screen a panel of 70 compounds for potential neurotoxins. Additionally, vascularized organoids were generated from Rett Syndrome (RTT) and MeCP2 duplication (M2) patients and differences in their phenotypical, functional and metabolic characteristics were compared. RTT and M2 organoids were further treated with BDNF, IGF, gentamycin and a HDAC inhibitor to assess their efficacy in changing a diseased organoid towards a healthy phenotype.

III-2-313

Assessment of toxic substances in neural differentiation of human ESCs

Eui-Bae Jeung, Jae-Hwan Lee, Hee Young Kang, Changhwan Ahn, Seon Young Park, Jin Yong An and Bonn Lee

College of Veterinary Medicine, Chungbuk National University, Cheongju, South Korea
ebjeung@chungbuk.ac.kr

Pluripotent human embryonic stem cells (ESCs) are used in assessing neurotoxicity of a variety of pharmacological compounds using characteristics of neuronal differentiation. Human ESCs were exposed to five pharmacological compounds during neural differentiation up to 28 days. Cytotoxicity for three anticancer agents (cytosine arabinoside, 5-fluorouracil, and hydroxyurea), two immune suppressing agents (indomethacin and dexamethasone) and a negative control agent (ascorbic acid) was evaluated by CCK assay. Expression level of neural markers was examined by real-time PCR and immunocytochemistry. Three anticancer agents were assessed to strong toxicants and two immune suppressing agents were examined to moderate toxicants by inhibition in cell viability and area of triangular chart reflecting expression level of neural specific markers. Cytosine arabinoside diminished expression of NESTIN and TUBB3 in neural cells differentiated from human ESCs. These findings chemicals that could have an impact on the embryonic stage and relevance of pharmacological compounds to embryonic neurogenesis.

Reference

Hong, E. J., Choi, Y., Yang, H. et al. (2015). Establishment of a rapid drug screening system based on embryonic stem cells. *Environ Toxicol Pharmacol 39*, 327-338.

III-2-41

An intact insect embryo as assay for developmental neurotoxicity testing

Michael Stern, Sarah Frömbling, Gregor Bergmann, Karsten Bode and Gerd Bicker

Division of Cell Biology, University of Veterinary Medicine Hannover, Hannover, Germany
michael.stern@tiho-hannover.de

Developmental neurotoxicity (DNT) of chemicals poses a serious threat to human health worldwide. Most *in vitro* testing methods monitor rather simple toxicological endpoints, whereas the formation of a functional brain requires precisely timed navigation of axons within a complex tissue environment. We aim to address this complexity by monitoring defects in axonal navigation of pioneer axons of intact locust embryos after exposure to chemicals. Since axon guidance mechanisms are highly conserved, such an insect assay will be indicative for DNT potential in humans. Embryos are kept in culture overnight with test chemicals, followed by a viability assay and immunolabeling of pioneer neurons. Defects in axonal outgrowth and navigation of pioneer axons are detected via conventional fluorescence microscopy and 3D Scanning Laser Optical Tomography. Currently, the system is being calibrated against a range of positive and negative compounds with known DNT potential.

Funded by BMBF 031L0062A.

Reference

Eickhoff, R., Lorbeer, R. A., Scheiblich, H. et al. (2012). Scanning laser optical tomography resolves structural plasticity during regeneration in an insect brain. *PLoS One 7*, e41236.

III-2-488

Comparative transcriptome analyses and functional characterization of developing human and rodent primary neural progenitor cells for DNT testing

Stefan Masjosthusmann[1], Marta Barenys[1], Jenny Baumann[1], Daniel Becker[1], Rene Deenen[2], Ulrike Hübenthal[1], Jördis Klose[1], Karl Köhrer[2], Barbara Petzuch[2], Clara Siebert[1] and Ellen Fritsche[1]

[1]Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany; [2]Biological and Medical Research Center, Heinrich-Heine-University, Düsseldorf, Germany
Stefan.Masjosthusmann@iuf-duesseldorf.de

There is an urgent need for alternative methods allowing faster and cheaper developmental neurotoxicity (DNT) testing for regulatory purposes. Aggregated primary neural progenitor cells (NPC) growing as neurospheres can contribute to a DNT testing battery as they functionally mimic proliferation, migration and differentiation *in vitro*. Transcriptome analyses of developing human and rodent NPC revealed overrepresentation of GO categories involved in organ and tissue development and regulation of major neurodevelopmental processes. Despite conservation of GO categories across species, transcriptome changes are highly species-specific with only ~10% overlap of regulated genes. Results of computational gene-protein interactions disclosed signaling pathways guiding neurodevelopmental processes that were experimentally validated. These pathway-to-function analyses provide information on the application domain of NPC and will contribute to higher confidence in NPC for DNT *in vitro* testing.

Session III-3: Models of Brain Disorders and Disease

Co-Chairs
Tim Shafer, US EPA, Research Triangle Park, NC, United States
William Mundy, US EPA, Research Triangle Park, NC, United States

III-3-842

Development of a suite of assays to screen for developmental neurotoxicity

William R. Mundy
Integrated Systems Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States
Mundy.William@epa.gov

Because the potential molecular targets for chemical disruption in the developing nervous system are not completely defined, our laboratory has developed *in vitro* assays that measure chemical disruption of neurodevelopmental processes at the cellular level. These include proliferation, apoptosis, axonal and dendritic development and synaptogenesis. Medium throughput assays performed using high content imaging were validated using "training set chemicals" known to alter the individual neurodevelopmental process. To evaluate the ability of these assays, individually and collectively, to predict DNT, we have tested a set of over 50 "DNT reference chemicals" and negative controls. Concentration-response relationships are established for both the neurodevelopmental endpoint of interest (e.g. proliferation) as well as cell health, allowing for determination of the selectivity of each chemical on neurodevelopmental processes. Chemical effects were diverse in that some hit only a single endpoint, while others altered multiple endpoints. No single assay was predictive of DNT, but the battery of assays was able to discriminate between negative controls and DNT reference compounds.

This abstract does not necessarily reflect U.S. EPA policy.

III-3-218

Development the assay of spontaneous activity in rat hippocampal neural networks

Daiju Yamazaki[1], Kazue Goto[1], Noriko Koganezawa[2], Kenji Hanamura[2], Tomoaki Shirao[2], Yuko Sekino[3] and Yasunari Kanda[1]
[1]National Institute of Health Sciences, Tokyo, Japan; [2]Department of Neurobiology and Behavior, Gunma University, Maebashi, Japan; [3]Laboratory of Chemical Pharmacology, University of Tokyo, Tokyo, Japan
daiju-y@nihs.go.jp

Thousands of compounds in the environment have not been characterized for developmental neurotoxicity (DNT) hazard. To address this issue, it is necessary to screen compounds rapidly for DNT hazard evaluation. Recently, multi-well microelectrode array (MEA) formats have become available to address the need for increased throughput. Here, we evaluated a protocol for spontaneous network activity using rat hippocampal neurons. Spontaneous network activity was increased in time-dependent manner and maintained between days 14 and 19, when 50,000 cells per 10 μl were seeded on electrodes in each well. Burst and synchronized spikes on several electrodes were observed on day 10. Moreover, we obtained similar results which all electrodes activated within 9 days at the latest in several experiments. In order to characterize for DNT hazard of compounds, we are evaluating positive- and negative-control compounds.

III-3-843

Using cortical cultures grown on microelectrode arrays to screen compounds for potential developmental neurotoxicity based on changes in network formation

Timothy J. Shafer, Christopher L. Frank, Jasmine P. Brown and Kathleen Wallace
Integrated Systems Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States
Shafer.Tim@epa.gov

Characterization of developmental neurotoxicity (DNT) hazard lags behind that of other adverse outcomes (e.g. hepatotoxicity) for thousands of compounds due the cost, time and number of animals needed to characterize DNT hazard. Thus, faster, less expensive approaches for DNT testing are needed. To address this, we have developed a microelectrode array (MEA)-based assay to screen for chemical effects on neural network formation. Primary cortical neurons grown on MEAs spontaneously form inter-connected networks, this allows spatial and temporal measurement of action potential spikes and bursts in these developing networks, and assessment of chemical effects on network formation. We screened a set of 60 compounds known to cause developmental neurotoxicity i*n vivo*, 49 of which altered network development. By comparing the potency of effects on network function to cell viability, compounds can be prioritized for additional testing based on the specificity of effects on network formation.

This work was supported by the US Environmental Protection Agency.
This abstract does not reflect U. S. EPA policy.

III-3-615

Dopaminergic cell recovery in an *in vitro* 3D model to study Parkinson's disease

Georgina Harris[1], Melanie Eschment[2], Helena Hogberg[1], David Pamies[1], Dana Freeman[3], Alexandra Maertens[1], Thomas Hartung[1,2] and Lena Smirnova[1]

[1]Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing, Baltimore, MD, United States; [2]University of Konstanz, Konstanz, Germany; [3]Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States
georgina.hlopez@gmail.com

To date, most *in vitro* toxicity testing focuses on acute effects of compounds at high concentrations. This testing strategy does not reflect real-life exposures contributing to long-term disease outcome. We use a 3D-human, dopaminergic, *in vitro* model to determine whether acutely-induced molecular effects are permanent or reversible and identify recovery and/or long-term disease mechanisms. We analyzed the effects of rotenone, a known Parkinson's inducer, after acute exposure and 7 days after compound removal. We identified irreversible (SNCA, OPTN and PINK1) and reversible gene regulation (ATF4, ATP50 and KEAP1). Short-term effects on neurite outgrowth and ATP were observed, however, at low concentrations cells were able to recover after wash-out. To study cellular neuroprotection, cells were exposed again after wash-out. Pre-exposed cells showed higher metabolic activity than controls (resilience) and differences in gene expression. We aim to further study the epigenetic changes involved in recovery and resilience. This is the first study showing the complexity of delayed effects after compound removal and re-exposure *in vitro*.

III-3-833

Development of an *in vitro* screening approach to identify potential developmental neurotoxicants/ neurotoxicants

Mamta Behl[1], Fred Parham[1], Jui-Hua Hsieh[1], Oksana Sirenko[2], Kristen Ryan[1], Nisha Sipes[1], Johannes Delp[3], Marcel Leist[3,4] and Raymond R. Tice[1]

[1]NIH/NIEHS, Research Triangle Park, NC, United States; [2]Molecular Devices, Sunnyvale, LLC, CA, United States; [3]Doerenkamp-Zbinden Chair of in-vitro Toxicology and Biomedicine, University of Konstanz, Konstanz, Germany; [4]CAAT-Europe, University of Konstanz, Konstanz, Germany
mamta.behl@nih.gov

With the recent increase in the prevalence of developmental neurotoxicity (DNT) in children and neurotoxicity (NT) in adults, reliable and efficient screening tools are needed to better evaluate, prioritize, and identify potential DNT/NT compounds. To date, there has been very little effort on assessing the potential DNT/NT hazard of the tens of thousands of compounds in commercial use. To develop a screening battery for DNT/NT compounds, the NTP and its collaborators are evaluating multiple assays spanning different domains of DNT/NT using an 80-compound library that includes compounds with known DNT or NT activity as well as environmental compounds of concern with unknown DNT/NT potential. To compare the relative activity of compounds across the different *in vitro* cell-based assay platforms, we used a point-of-departure (POD) method to set assay-based thresholds. This talk summarizes the results obtained using these different platforms and compares the findings across platforms and with the results of the Tox21/ToxCast quantitative high throughput screening assays. Finally, *in vitro-in vivo* extrapolation (IVIVE) with a subset of compounds demonstrated was performed to correlate the POD in the *in vitro* models with PODs in guideline DNT studies in rodents.

III-3-410

Computational modeling of the neurovascular unit to predict microglia mediated effects on blood-brain barrier formation

Todd Zurlinden[1], Katerine Saili[1], Andrew Schwab[2], Aymeric Silvin[3], Sid Hunter[2], Nancy Baker[4], Richard Spencer[5], Florent Ginhoux[3] and Thomas Knudsen[1]

[1]ORD/NCCT, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States; [2]ORD/NHEERL/ISTD, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States; [3]Agency for Science, Technology and Research (A*STAR), Singapore Immunology Network, Singapore; [4]NCCT, Leidos, Research Triangle Park, NC, United States; [5]NCCT, ARA, Research Triangle Park, NC, United States
zurlinden.todd@epa.gov

Development of the neurovascular unit (NVU) involves interactions between endothelial cells, pericytes, neuroprogenitor cells, and microglia. We constructed an *in silico* model of the developing neuroepithelium in CompuCell3D which recapitulated a suite of critical signaling pathways (Notch/dll4, CSF-1, VEGF-A/C) and cellular behaviors (growth, migration, proliferation, differentiation, apoptosis). Imputing ToxCast *in vitro* profiling data into the simulated neuroepithelium enabled predictions of developmental neurovascular toxicity. For example, targeting CSF-1R *in silico* yielded a quantitative effect on microvascular arborization. Cybermorphs can now be qualified against *in vivo* phenotypes from CSF-1R ablation genetically or immunologically. The *in silico* models, combined with *in vitro* cell-level data, can guide engineering of human cell-based NVU-devices to prioritize untested environmental chemicals for further testing.

This abstract does not reflect U.S. EPA policy.

Session III-4: New Advances in Models for Skin

Co-Chairs
Nathalie Alépée, L'Oréal, Aulnay sous Bois, France
Grace Patlewicz, US EPA, Research Triangle Park, NC, United States

III-4-191

In vitro skin microtissue model for toxicology and efficacy testing

Simon Stroebel, Aparna Neelakandhan, Nicole Buschmann and Jens Kelm
InSphero AG, Schlieren, Switzerland
simon.stroebel@insphero.com

Transwell skin models, standard for conducting skin safety and efficacy studies, show limitations in assay up-scaling and include a manual manufacturing process which can resulting in inconsistent assay readouts.

We present a spherical skin microtissue model consisting of a primary fibroblast-rich dermal core surrounded by differentiating keratinocytes to from epidermal tissue. The dermal tissue compartment, rich in collagen type I/III, show high structural similarities to native dermis. The epidermis separates in proliferating keratinocytes in the basal layer and differentiating cells in the suprabasal layers. Peripheral cells are terminally differentiated and define a cornified layer with a penetration barrier. LDH-leakage test on models with differentiated epidermal structures was significantly reduced compared to tissue models which consists of dermal fibroblasts only.

The miniaturized skin model allows for uniform production and assay up-scaling to increased readout reproducibility.

III-4-62

Use of *in vitro* methods according to OECD GD 129 and OECD TG 439 to classify children's articles aligned with the UN GHS principles

Renata Damiani[1], Thais Feitosa[1], Vanja Dakic[2], Josemar Silva[3], Tatiana Cunha[1,4], Marcos Noronha[1,4], Olga Higa[1,5], Nathalie Alépèe[6], Christian Pellevoisin[7], Mariano Bacellar[4], Luciene Balottin[3], Rodrigo De Vecchi[2] and Fabiana Medeiros[1,4,5]
[1]GLP Test Facility on Research and Innovation of Vitro Methods, Biosintesis Laboratory, São Paulo, Brazil; [2]Research and Innovation, L'Oréal Research and Innovation, Rio de Janeiro, Brazil; [3]Brazilian National Alternative Methods Network (RENAMA), National Institute of Metrology, Quality and Technology (INMETRO), Rio de Janeiro, Brazil; [4]Schools Articles Safety Brazilian Technical Committee (CEE 102), Brazilian Association of Technical Standards (ABNT), São Paulo, Brazil; [5]Biotechnology Department, Nuclear and Energy Research Institute (IPEN/CNEN-SP), São Paulo, Brazil; [6]Research and Innovation, L'Oréal Research and Innovation, Aulnay sous Bois, France; [7]Research and Innovation, Episkin Academy, Lyon, France
renata.damiani@biosintesis.com.br

Schools Articles Safety Brazilian Technical Committee (TC/ABNT CEE 102) has taken up the challenge to update the national safety standard ABNT NBR 15236:2016 that complies with the regulations and guidelines on alternatives testing. The current aim was to provide an *in vitro* strategy for risk assessment of school articles with data integration following OECD GD129 & TG439. *In Vitro* Acute Toxicity and SkinEthic™ Reconstructed Human Epidermis Skin Irritation methods were adapted from medical devices protocols by measuring cytotoxic effects of extracts prepared according ISO10993. Results obtained on > 150 school articles (pens, pencils, crayons, watercolor, adhesives, correction fluid) were reproducible. 100% concordance with UN GHS classification for discriminating approval (95%) versus non-approval (5%) was observed. In conclusion, these results are important to ensure that regulatory authorities in Brazil are provided with the appropriate data required for decision-making.

References

ABNT NBR (2016). No. 15236. Schools Articles Safety.
OECD (2010). GD No. 129. Cytotoxicity tests to estimate starting doses for Acute Oral Systemic Toxicity.
OECD (2015). TG No. 439. In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method.

III-4-504

The potential of protein reactivity to predict skin sensitizing potency: Of peptide depletion, reaction time and tested concentrations

Britta Wareing[1], Susanne Kolle[1], Daniel Urbisch[1], Dennis Mulliner[2], Bennard van Ravenzwaay[1] and Robert Landsiedel[1]

[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany; [2]Computational Chemistry and Biology, BASF SE, Ludwigshafen, Germany

britta.wareing@basf.com

While skin sensitization hazard can already be assessed using non-animal methods, the classification of their potency (GHS sub-categories 1A and 1B) was not yet attained (Basketter et al., 2015). Since the protein-adduct formation determines the dose of the allergen in the skin, peptide reactivity was used to assess the potency. The Direct Peptide Reactivity Assay (DPRA; one concentration, one time-point) provided an adduct yield which did not sufficiently discriminate between sub-categories 1A and 1B. The "quantitative DPRA" (several concentrations, one time-point), discriminated sub-categories with a higher accuracy. Finally, the "kinetic DPRA" (several concentrations and time-points) was used to approximate the rate constant of the Cys-peptide-adduct formation. 35 of 38 skin-sensitizing substances were correctly assigned to their potency sub-categories. These results indicate that the kinetic DPRA may be the missing puzzle piece to fully replace *in vivo* testing for assessing skin sensitization.

Reference

Basketter, D., Ashikaga, T., Casati, S. et al. (2015). Alternatives for skin sensitisation: Hazard identification and potency categorisation: Report from an EPAA/CEFIC LRI/Cosmetics Europe cross sector workshop, ECHA Helsinki, April 23rd and 24th 2015. *Reg Tox Pharm 73*, 660-666.

III-4-500

L'Oréal's stacking meta-model for skin sensitization: Assuring consumer safety

Aurélia Del Bufalo, Silvia Teissier, Nathalie Alepée and Jacques Clouzeau

L'Oréal, Research & Innovation, Aulnay sous Bois, France

adelbufalo@rd.loreal.com

Skin sensitization is a major environmental and occupational health hazard. Since March 2013, the 7th Amendment of the Cosmetics Directive prohibits in Europe the marketing of cosmetic products containing new ingredients which were tested on animal-based assays.

While there is a common understanding of the Adverse Outcome Pathways (AOP) leading to skin sensitization, as well as an OECD initiative on the implementation of Integrated Approaches to Testing and Assessment (IATA), the ways of applying such data to allow risk assessment of new ingredients is still in its early experimental phase.

Our own integrated testing strategy was developed using a statistical stacking meta-model. Based on case studies, we will illustrate how non-sensitizers can be predicted with a high degree of confidence and we will present the ongoing refinements on potency to reach the final goal of safety evaluation of new ingredients.

III-4-579

Analysis of fragrance ingredients in Sens-IS assay for skin sensitization

Rahul Parakhia, Devin O'Brien and Anne Marie Api

Research Institute for Fragrance Materials, Inc. (RIFM), Woodcliff Lake, NJ, United States

rparakhia@rifm.org

Regulatory and public pressure has accelerated the development and validation of *in vitro* tests to determine the skin sensitization potential of chemicals. In addition to the ARE family of genes, another set of genes (the so-called Sens-IS) has been recently associated with skin sensitization. The Sens-IS test is based on the analysis by RT-PCR (reverse transcription polymerase chain reaction) of three sets of genes, a set of 23 genes reflecting the irritant potential and sets of 17 ARE and 21 Sens-IS genes, measuring sensitization potential of a material. We report data and analysis of 75 fragrance ingredients in the Sens-IS test method. For hazard identification, compared to animal methods, Sens-IS correctly captured 54 of the 64 sensitizers, and 7 of the 10 non-sensitizers. A comparison of the potency between Sens-IS and animal methods is also presented. We show that Sens-IS is another alternative *in vitro* test which can detect sensitization potential of fragrance ingredients.

III-4-769

Mixture used in permanent hair dyes enhances allergic parameters in reconstructed epidermal equivalents

Thalita Boldrin Zanoni[1], Sander Spiekstra[2], Silvia Berlanga de Morais Barros[1], Sue Gibbs[2] and Silvya Stuchi Maria-Engler[1]

[1]Skin Biology Group, University of São Paulo, São Paulo, Brazil; [2]Departments of Dermatology, VU University Medical Centre, Amsterdam, The Netherlands

thalitaz@fcfrp.usp.br

Skin is a frequent target of allergic reactions caused by topic exposure of permanent hair dyes. These hair dyes are formed by a mixture of ingredients that vary from low to extreme skin sensitizers that react among them forming unknown by-products. To evaluate the allergenic potential of these dyes p-phenylenediamine (PPD), Resorcinol (RES), H_2O_2 alone and after combination were topically exposed to an *in house* reconstructed epidermal equivalent (EEQ) that structurally and functionally resembles human epidermis. Next, some parameters involved in skin allergy such as epidermal viability, barrier loss, and IL-1α were evaluated. Our data indicates that the ingredients alone do not alter evaluated parameters while exposure to the mixture of PPD/H_2O_2/RES and PPD/H_2O_2 leads to morphological changes, barrier loss, apoptotic cells increase followed by increase of IL-1α. Therefore, the formation of the by-products could be the key of skin allergic induction and should be better investigation.

III-4-729

An *in vitro* human skin test for assessing allergy and sensitization

Shaheda Ahmed[1,2], *Louis Bibby*[1,2], *Asif Tulaf*[1,2] *and Anne Dickinson*[1,2]

[1]Alcyomics Ltd, Newcastle, United Kingdom; [2]Newcastle University, Newcastle, United Kingdom
s.s.ahmed@ncl.ac.uk

There are currently limited reliable human *in vitro* assays which test for sensitisation, immunogenicity and efficacy of novel compounds that are equivalent to *in vivo* animal testing. Here we describe a novel test named Skimune™ , developed as a non-artificial (non-3D) human *in vitro* assay which can predict adverse immune reactions. The test uses blood and skin biopsies taken from healthy volunteers and gives a predictive readout of skin damage which correlates with inflammatory cytokine release and T cell proliferation responses. The data from the different assays is integrated to provide a precise report of the potential risk of the test compound to induce adverse reactions and thus allows the study of immune responses in the presence of chemicals or cosmetics and drugs such as monoclonal antibodies, biologics or small molecule drugs.

Skimune™ was shown to correctly predict the sensitising capacity of chemicals with 95% sensitivity, 95% specificity and 0.96 correlation to the gold standard mouse local lymph node assay (mLLNA). Additionally, used as a preclinical tool it can correctly predict allergic responses to new therapeutics before testing in man. Ten antibody formulations were tested including TGN1412 analogue with promising results. The test could have predicted the serious life-threatening cytokine storm which affected healthy volunteers in the 2006 Northwick Park trial. The test allows for early detection of adverse events allowing improved development of therapeutic drugs and compounds and aid in the safety profiling of compounds.

Reference

Ahmed, S. S., Wang, X. N., Fielding, M. et al. (2016). An in vitro human skin test for assessing sensitization potential. *J Appl Toxicol 36*, 669-684.

Session III-5: New Models for Cardiovascular Function, Physiology and Disease

Co-Chairs
Ivan Rusyn, Texas A&M University, College Station, TX, United States
Johan Heemskerk, Maastricht University, Maastricht, The Netherlands

III-5-179

A population-based organotypic human *in vitro* model for cardiotoxicity testing

Ivan Rusyn, Fabian Grimm and Weihsueh Chiu
Texas A&M University, College Station, TX, United States
irusyn@tamu.edu

The implementation of organotypic culture models in human health safety assessments is impeded by the lack of multidimensional high-throughput testing strategies that are amenable for (1) providing chemical-specific uncertainty factors for estimation of inter-individual susceptibilities to adverse chemical effects and (2) for extrapolation of *in vitro*-derived dose-response relationships to physiologically-relevant exposure levels. We previously demonstrated the feasibility of combinatorial *in vitro*/ *in silico* screening approaches for functional and mechanistic cardiotoxicity profiling in human induced pluripotent stem cell (iPSC)-derived cardiomyocytes, thereby prompting the hypothesis that a population-based *in vitro* cardiotoxicity model is a viable option for translatable chemical hazard assessment that is amenable for data-integrative *in vitro*-to-human *in vivo* extrapolation. Thus, we exposed cardiomyocytes derived from 30 "healthy" donors to more than 100 chemicals representing a broad range of toxicologically relevant compound classes, i.e. drugs and environmental chemicals, including pharmaceuticals with known human cardiophysiological concentration-response profiles, allowing either direct extrapolation or model-derived prediction of human cardiotoxic effects. Cells were exposed to test chemicals in concentration-response covering nanomolar and micromolar concentration ranges, selected to be representative of human C_{max} range (where available) for 90 min and 24 hrs. Kinetic Ca^{2+} flux measurements and high-content live cell imaging revealed chemical class-specific effects on cardiomyocyte contractility and cellular/ mitochondrial toxicity. Variation in phenotypic responses to chemical treatments indicated biologically-relevant, inter-individual variability to chemical exposure. Likewise, untreated iPSC cardiomyocytes exhibited donor cell-specific differences in cardiophysiologic performance. Altogether, this work is pioneering the "diversity in a dish" concept through the use of organotypic cell culture models for human health safety assessments.

III-5-472

Robust fluidic physiomimetic platforms for cardiac and pancreatic microsystems

Ashutosh Agarwal
Department of Biomedical Engineering, Department of Pathology & Laboratory Medicine, University of Miami, Miami, FL, United States
a.agarwal2@miami.edu

We report the design and fabrication of a robust fluidic platform built out of inert plastic materials and micro-machined features that promote optimized convective fluid transport. A coupled quantitative fluid dynamics computational model was first utilized to design device geometries that are optimal for complete perfusion of three-dimensional islets and cardiac spheroids, effective collection of secreted insulin, and minimization of system volumes and associated delays. Fluidic devices were then fabricated through rapid prototyping techniques, such as micromilling and laser engraving, as two interlocking parts from materials that are non-absorbent and inert. The optimized design of convective fluid flows, use of bio-inert and non-absorbent materials, reversible assembly, manual access for loading and unloading of 3D cultures, and straightforward integration with commercial imaging and fluid handling systems proved to be critical for perfusion assay, and particularly suited for time-resolved optogenetics studies.

Reference

Lenguito, G., Chaimov, D., Weitz, J. et al. (2017). Resealable, optically accessible, PDMS-free fluidic platform for ex vivo interrogation of pancreatic islet. *Lab Chip 17*, 772-781.

III-5-774

Translating human iPSC-cardiomyocyte disease and toxicity models from the bench to the bedside

Blake Anson

Cellular Dynamics International, Madison, WI, United States
banson@cellulardynamics.com

Human induced pluripotent stem cell (iPSC)-cardiomyocytes have moved from a laboratory phenomenon to a (nearly) mainstream experimental model for basic and applied biological investigations. Recent advances have demonstrated the advantages and utility in replacing animals and non-human cell models with iPSC-cardiomyocytes across a variety of toxicity testing paradigms and disease models. This talk will begin with an overview of the basic physiology and function of iPSC-cardiomyocytes under current and cutting-edge cell culture methods and move into discussing how the model(s) are being used to; (1) predict pro-arrhythmia and contractility issues, (2) detect cardiac side effects of small molecule and biologic oncology treatments, and (3) represent induced and innate disease models of cardiac hypertrophy and diabetic cardiomyopathy.

III-5-379

In vitro thrombosis models

Johan W. M. Heemskerk[1], Johanna P. van Geffen[1], Constance C. F. Baaten[1], Magdolna Nagy[1], Susanne de Witt[1], Sanne L. N. Brouns[1], Bibian M. E. Tullemans[1], Frauke Swieringa[1,2], Marijke J. E. Kuijpers[1] and Paola E. J. van der Meijden[1]

[1]Maastricht University, Maastricht, The Netherlands; [2]Leibnitz Institute ISAS Dortmund, Dortmund, Germany
jwm.heemskerk@maastrichtuniversity.nl

Thrombosis, arterial and venous, is still the number one causes of death in the Western world. The thrombotic process in arteries and veins is triggered by vascular damage or flow perturbations, for example caused by atherosclerotic plaque rupture. The thrombotic process, leading to vascular occlusion, is instigated by aggregation of blood platelets, platelet procoagulant activity, and formation of a fibrin clot. Supporting roles are provided by neutrophils and by red blood cells, which are massively trapped into the fibrin network. *In vivo* mouse models have shown that: *(i)* > 350 genes (encoding for platelet, plasma or vascular proteins) are implicated in arterial thrombus formation; *(ii)* collagen and tissue factor are the main vascular triggers of the thrombotic process; *(iii)* thrombus instability is a key regulatory mechanism for preventing permanent occlusion; and *(iv)* inflammation contributes to thrombosis in a variable degree, with increased importance in stroke models.

In the past decade, our and other laboratories have developed microfluidic devices for 3R approaches to assess the thrombotic process *in vitro* (De Witt et al., 2014; Nagy et al., 2017). Herein, isolated whole blood is flowed perfused over micro-arrays of thrombogenic surfaces at defined wall-shear rates, mimicking those of arteries or veins. The results so far have shown that animal thrombosis research to a major degree can be: *(r1)* replaced by the use of human blood instead of mouse blood; *(r2)* reduced due to the small blood volumes needed; and *(r3)* refined by multiparameter measurements in the flow-chip of platelet thrombus and fibrin clot formation.

The use of microfluidic devices has shown that, as *in vivo*, immobilized collagen and tissue factor are key triggers of the thrombotic process. Collagen appears to have a relatively high activity at high, arterial shear rates, by binding von Willebrand factor and activating platelets via GPVI. The role of tissue factor in promoting fibrin formation relatively increases at low, venous shear rates via stimulation of the extrinsic coagulation pathway. Furthermore, the flow and shear rates also determine the kinetics of thrombus buildup and stabilization.

Application of collagen-coated microfluidic devices with mouse blood showed a good correlation with results from common models of $FeCl_3$- or ligation-induced arterial thrombosis (n = 35 genes, $P < 0.05$). Multiparameter assessment of blood samples from patients with a prothrombotic propensity (atherosclerosis, peripheral arterial disease, cancer) displayed a relative gain-of-function in collagen-dependent buildup of platelet thrombi. Flow assay conditions can be adapted to optimally record the thrombus-suppressing effects of antiplatelet or anticoagulant medication. Multiparameter assaying of blood from patients with mutations linked to platelet or coagulant dysfunctions revealed sub-processes in thrombus formation that associated with specific platelet dysfunctions or coagulation morbidities.

References

De Witt, S. M., Swieringa, F., Cavill, R. et al. (2014). Identification of platelet function defects by multi-parameter assessment of thrombus formation. *Nat Commun 5*, 4257.

Nagy, M., Heemskerk, J. W. and Swieringa, F. (2017). Use of microfluidics to assess the platelet-based control of coagulation. *Platelets*, in press.

III-5-353

Long-term survival of spontaneously beating cardiomyocyte spheroids a 2-Organ-Chip (2-OC) platform

Talita Marin[1], Murilo Carvalho[1], Diogo Biagi[2], Marcos Valadares[2], Nathalia Indolfo[1] and Eduardo Pagani[1]

[1]LNBio/CNPEM, RENAMA, Campinas, Brazil; [2]PluriCell Biotech, São Paulo, Brazil
talita.marin@lnbio.cnpem.br

The use of human primary cardiomyocytes (hCM) for cardiotoxicity depends on availability and have poor consistency. Cardiac cell lines phenotype differs from primary hCM. Pluripotent human steam cells differentiated into cardiomyocytes (HiPSC-CM) are available and have functional and morphological properties closer to hCM. Most groups report HiPSC-CM survival for no longer than 14 days. We produced HiPSC-CM spheroid aggregates of 300 to 600 μM and keep them alive and continuously beating at 30 to 40 bpm for 70 days in the 2-OC (by TissUse GmbH). At the 70th day, the still beating heart organoids were fixed and stained with OsO4. Electron microscopy showed functional sarcomeres and mitochondria in the HiPSC-CM from organoids. X-ray 3D morphology (synchrotron micro-tomography) showed homogeneous cellular distribution with few discontinuities and few necrotic centers in the core. These results show that microfluidic chips may improve the functionality and survival of HiPSC-CM organoids.

References

Denning, C., Borgdorff, V., Crutchley, J. et al. (2016). Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform. *Biochim Biophys Acta 1863*, 1728-1748.

de S e Silva, J. M., Zanette, I. and Noël, P. B. (2015). Three-dimensional non-destructive soft-tissue visualization with X-ray staining micro-tomography. *Sci Rep 5*, 14088. doi:10.1038/srep14088

Hendriksen, C., Baumans, V. and Blaauboer, B. (2012). Animals are more than tools. *Altern Lab Anim 40*, 235-257.

III-5-812

Preclincial cardiac safety testing using hIPSc-CM test systems and the impact on 3Rs

Khuram Chaudhary
Safety Pharmacology, GlaxoSmithKline, King of Prussia, PA, United States
khuram.w.chaudhary@gsk.com

The investigation of untoward effects of drugs is critical to bringing safe medicines to market. ICHS7A/B guidelines are used to assess preclinical safety of drugs on the cardiovascular system, to mitigate potential cardiotoxicity. While both *in vivo* and *in vitro* test systems are considered complimentary, the use of novel technologies is highly encouraged. The advent of stem cell derived cardiomyocytes (hIPSc-CMs), has revolutionized the field of cardiotoxicity testing. In fact, a novel paradigm for preclinical testing, the Comprehensive *In vitro* Proarrhythmia Assay, has been proposed and endorsed by regulators to shift the investigation of cardiotoxicity earlier in drug development by exploiting advances in hIPSc-CM assays, *in silico* modeling and standardized ion channel pharmacology studies. In conjunction with innovations in bioengineering and engineered heart tissue constructs, it's clear that increased emphasis on *in vitro* assays, has the potential to positively impact the 3Rs – replacement, reduction and refinement of animal use. We will review the landscape of 2D/3D hIPSc-CM assays, in comparison to accepted cardiac test systems.

Session III-6: Models of Developmental and Reproductive Biology

Co-Chairs
Thomas Knudsen, US EPA, Research Triangle Park, NC, United States
Xiaozhoung (John) Yu, University of Georgia, Athens, GA, United States

III-6-782

Predictive modeling of developmental toxicity with pluripotent stem cells

Thomas Knudsen
National Center for Computational Toxicology, US EPA, Research Triangle Park, United States
knudsen.thomas@epa.gov

The developing embryo is a complex adaptive system capable of self-organization but vulnerable to genetic and environmental perturbation. Recapitulating this complexity poses a challenge for alternative *in vitro* platforms. The devTOX quick Predict platform (Stemina) is an *in vitro* human pluripotent stem cell-based assay used to assess a wide range of chemicals for potential developmental toxicity. We screened 1065 ToxCast chemicals in this assay for an exposure-based potential for developmental toxicity and entered the data into the ToxCast pipeline. Performance models range from 87-91% balanced accuracy for reference compounds and positive calls on 177 chemicals. These results will contribute to a predictive modeling tool set for screening and mechanistic modeling of developmental toxicity that addresses chemical effects on morphological development in a more quantitative way.

This abstract does not reflect U.S. EPA policy.

III-6-175

Evaluation of an adherent mouse embryonic stem cell *in vitro* assay to predict developmental toxicity of ToxCast chemicals

Susan Jeffay, Harriette Nichols, Maria Hoopes, Mitch Rosen, Marianne Barrier and Sid Hunter
US Environmental Protection Agency, Research Triangle Park, NC, United States
jeffay.susan@epa.gov

The potential for most environmental chemicals to induce developmental toxicity is unknown. Mouse embryonic stem cell (mESC) assays are an alternative *in vitro* model, hence, comparisons between chemical effects in mESCs and *in vivo* are needed. An adherent mESC assay was used to evaluate ToxCast Phase I and II chemicals at 4 concentrations and 2 time points. Cell cultures were evaluated for cell number and differentiation: on culture day 4, the gastrulation biomarker goosecoid (GSC) and on culture day 9, the cardiomyocyte biomarker myosin heavy chain (MHC) were used. At day 4 (1078 total chemicals), 114 affected GSC, 95 cell number and 173 both endpoints. At day 9 (320 total chemicals), 34 affected MHC, 111 cell number and 60 both endpoints. For chemicals affecting mESCs at both time points, 68% are reported as developmental toxicants in EPA's Toxicity Reference Database. mESCs can be used to rank/prioritize potential developmental toxicants.

This abstract does not reflect U.S. EPA policy.

III-6-698

An animal-free *in vitro* three-dimensional testicular cells co-culture model for evaluating male reproductive toxicants

Xiaozhong (John) Yu, Lei Yin and Shenxuan Liang
University of Georgia, Athens, GA, United States
yuxz@uga.edu

Primary testicular cells co-culture models have been used to evaluate testicular abnormalities during development, and was able to identify testicular toxicity of phthalates. However, primary testicular cells co-culture model has its disadvantage in employing animals for the isolation of testicular cells and complicated isolation procedure leads to inconsistency results. We developed and constructed an *in vitro* testicular co-culture model from rodent testicular cell lines, including spermatogonial cells, Sertoli cell, and Leydig cell with specified cell density and Extracellular Matrix (ECM) composition. Using comparative high-content analysis of F-actin cytoskeleton structure between the co-culture and single spermatogonial cell culture, we demonstrated a three-dimensional structure of the co-culture, which created an *in vivo*-like *niche*, and maintained and supported germ-line functioning within a three-dimensional environment. We validated this model by discriminating between reproductive toxicants and non-toxicants among 32 compounds in comparison to the single cell culture models. Furthermore, we conducted a comparison between the *in vitro* (IC_{50}) and *in vivo* reproductive toxicity testing (lowest observed adverse effect level on reproductive system, rLOAEL). We found the *in vitro* co-culture model was able to classify the tested compounds into four clusters, and identify the most toxic reproductive substances, with high concordance, sensitivity, and specificity at 78.57%, 81.11%, and 80%, respectively. We observed a strong correlation of IC_{50} from the *in vitro* testicular co-culture model with the *in vivo* testing results. Our results suggest that this novel *in vitro* co-culture model may be useful for screening testicular toxicants and prioritize chemicals for further assessment in future.

This work was supported by the CDC NIOSH R21 OH 010473,NIEHS R43ES027374 and Alternatives Research & Development Foundation (ARDF).

III-6-63

An engineered cell-instructive stroma for the fabrication of a novel full thickness human cervix equivalent *in vitro*

Vincenza De Gregorio[1], Giorgia Imparato[1], Francesco Urciuolo[1], Maria Lina Tornesello[2], Clorinda Annunziata[2], Franco Maria Buonaguro[2] and Paolo Antonio Netti[1]
[1]Center for Advanced Biomaterials for Healthcare, Istituto Italiano di Tecnologia, Napoli, Italy; [2]Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione Pascale", Napoli, Italy
eziad35@gmail.com

For the first time, it is reported the production of an organotypic cervical model featured by a scaffold-free stromal tissue resembling the ECM composition and organization of the native counterpart as well as a well-differentiated epithelium. To reach this aim, human cervical microtissue precursors were produced, characterized and used as functional building units to fabricate a cell-synthesized cervical stroma equivalent by means of a bottom-up approach. Several analyses reveal the extent of fundamental epithelial biomarkers and the presence of ECM molecules, demonstrating that the natural tissue architecture of cervical tissues was reproduced. Our results suggest that the bottom-up technology used to produce these three-dimensional human cervical equivalents provides a fully-functional organotypic cervical model that may be used as a valuable tool to investigate the epithelial-stromal interactions and for testing new therapeutics *in vitro*.

References

Imparato, G., Urciuolo, F., Casale, C. and Netti, P. A. (2013). The role of microscaffold properties in controlling the collagen assembly in 3D dermis equivalent using modular tissue engineering. *Biomaterials 34*, 7851-7861.

Tornesello, M. L., Losito, S., Benincasa, G. et al. (2011). Human papillomavirus (HPV) genotypes and HPV16 variants and risk of adenocarcinoma and squamous cell carcinoma of the cervix. *Gynecol Oncol 121*, 32-42.

Session III-7: Models for Liver Function and Disease

Co-Chairs
Terrance Kavanagh, University of Washington, Seattle, WA, United States
Jeetu Eswaraka, Amgen, Thousand Oaks, CA, United States

III-7-825

Mechanistic insight of human drug-induced liver injury

Alison Vickers
Human Translational Models LLC, Irvine, CA, United States
aemvickers@me.com

Liver organ slices aid to define the dose-response and mechanism of drug-induced injury, as well as to define species differences. These organotypic cultures maintain the 3-dimensional multicellular complexity and architecture to mimic *in vivo* function. Combining gene expression with cellular functional measurements and histopathology successfully characterizes the molecular mechanisms of drug action on key pathways that are causal to organ dysfunction and injury. Various toxicities detected in the animal studies is investigated in animal and human organ slices to define the relevance for human, to define the concentration which will induce the potential side-effect, and to identify potential biomarkers. Examples will be presented on drugs linked with oxidative stress and mitochondrial dysfunction, hemolysis, and a comparison of several drugs associated with clinical liver effects.

III-7-285

The C-DILI assay: A new *in vitro* method to predict chemically-induced liver toxicity

Jim McKim[1], Jonathan Jackson[2], Chris Black[2], Kimberly Freeman[2] and Kenneth Brouwer[2]
[1]IONTOX, LLC, Kalamazoo, MI, United States; [2]Qualyst Transporter Solutions, LLC, Durham, NC, United States
jmckim@IONTOX.COM

A major concern for chemical and cosmetic companies is the potential for chemicals to cause liver injury in humans. The C-DILI Assay is a novel *in vitro* method that integrates multiple components of the Adverse Outcome Pathways for cholestasis. The assay evaluates a compound's potential to induce liver injury caused by accumulation of bile acids (e.g. chemicals that are bile acid disruptors). The C-DILI Assay integrates multiple compound effects including BSEP inhibition, inhibition of basolateral efflux transporters (OSTα/βand/or MRP3/4) and FXR antagonism, all of which impact the levels of bile acids in the liver. A test set of 45 chemicals showed a sensitivity of 88%, a specificity of 97%, and an accuracy of 96% demonstrating a highly predictive assay.

III-7-841

Using 3D bioprinted human tissues to model complex liver disease and drug toxicity mechanisms

Thomas J. Murphy
Organovo, San Diego, CA, United States
TMurphy@organovo.com

The high rate of attrition among clinical-stage therapies underscores the need for *in vitro* testing models that more accurately recapitulate *in vivo* human biology. Human tissue biology is strongly influenced by the unique interplay and extensive cross talk that exists between different resident cell populations. These cell types are most often spatially arranged in a specific architecture which defines their biological function and mechanistic response to drug treatment over time. However, most conventional *in vitro* cell-based systems used to test candidate drugs lack these key biological features. Three-dimensional bioprinted tissues are able to successfully model this cellular complexity and therefore offer major advantages over conventional *in vitro* systems with respect to predictive modeling. Bioprinted tissues incorporate key architectural features and primary cell types which can be maintained in culture on a timescale of several days to weeks.

The primary goal of this session will be to educate participants on the bioprinting process and how this technology is currently being applied in the manufacture of several types of human tissues. Specific case studies will highlight the advantages of using bioprinted liver tissue to model aspects of liver disease such as NAFLD and fibrosis.

III-7-840

Human tissues and cells for basic and translational research: The path towards standardized practices

Edward L. LeCluyse

LifeSciences Division, Institute of Regenerative Medicine, Research Triangle Park, NC, United States
edward_lecluyse@lifenethealth.org

With the promise of new human-based culture technologies to reduce, refine, or replace animal use while minimizing risks of drug and chemical induced liver toxicity, there is a greater need for more reliable and well-defined sources of human tissues and cells. Historically, there has been a lack of standardized practices and well-defined methods for the recovery, preservation and characterization of human tissues for basic and translational research. This has been at the heart of some of the variability in quality and reproducibility, which has in turn led to a reluctance over the use of human tissues and cells for some scientific applications. As such, well-characterized human tissues and cells with respect to their biology, pathology and integrity are needed if we are to meet the promise and growing demand of these leading edge technologies to serve as human surrogate model systems for breakthroughs in our understanding of human disease and toxicity. However, there are a number of technical and scientific challenges that correspond to meeting industry-wide specifications if we are to successfully meet this growing trend. First, identifying and qualifying acceptable sources of human tissues and cells that meet the specifications of the biomedical and translational research communities. Second, providing a better definition of "what is normal" and "what is diseased" within the human population for key biological parameters. For each of these key parameters, recommendations and guidance need to be published for acceptance criteria, acceptable ranges, quality metrics and standards prior to use for biological, pharmacological or toxicological laboratory testing. Third, communication between the research communities and the organ and tissue procurement organizations needs to be improved in order to successfully meet the requirements for adopting best practices and establishing standardized methods. This presentation also will provide insights and guidance for scientists who wish to better understand the considerations and caveats when sourcing human tissues and cells for their research. It also stresses the importance of starting with good-quality, well-defined source material and highlights examples of how donor and recovery specifications impacts the validation and fidelity of *in vitro* model systems.

III-7-827

Risk assessment for cholestatic hepatotoxicity: Integrating transporter inhibition and FXR mediated regulation into a predictive *in vitro* assay

Kenneth Brouwer, Jonathan Jackson, Kimberly Freeman, Robert St. Claire and Chris Black

Qualyst Transporter Solutions, Durham, NC, United States
kennethbrouwer@qualyst.com

BSEP inhibition potency does not correlate with cholestatic drug-induced liver injury (DILI) severity. Bile acid (BA) concentrations are tightly regulated through synthesis, metabolism and transport. Under cholestatic conditions, an FXR mediated adaptive response initiates basolateral efflux of BAs via OSTα/β to reduce the intracellular concentration of BAs. Chenodeoxycholic acid (CDCA) activated FXR, decreased CYP7A1 expression, induced OSTα/β and BSEP in Transporter Certified™ sandwich-cultured human hepatocytes (SCHH). Gene expression changes were linked to functional changes in the total bile acid pool and B-CLEAR® technology was used to evaluate the hepatobiliary disposition of endogenous BAs. Assessment of a drug candidate's cholestatic DILI potential requires a fully integrated cell system capable of generating this adaptive response, and integrating the acute and chronic effects of the drug candidate on the hepatobiliary disposition of bile acids.

Session III-8: Models of the Renal System

Co-Chairs
Paul Jennings, Medical University of Innsbruck, Innsbruck, Austria
Edward Kelly, University of Washington, Seattle, WA, United States

III-8-742

Requirements of human *in vitro* models for repeat dose nephrotoxicity testing

Paul Jennings
Medical University of Innsbruck, Innsbruck, Austria
paul.other@gmail.com

Human models for *in vitro* nephrotoxicity testing include immortalised cells, telomerase overexpression, primary cells, embryonic stem cells and more recently human induced pluripotent stem cells (iPSC). All models are derived from different individuals, use different culture media and culture conditions and are utilised in varied states of proliferation and differentiation. While standardisation remains an issue, stability of the system over time is key for long term experiments. We have tested the human proximal tubule cell line (RPTEC/TERT1), and could demonstrate that after monolayer maturation it remains functionally stable for at least two weeks. Only very minor alterations in the transcriptome and metabolome were observed in control cells over this two-week period, whereas monolayers treated with nephrotoxins showed strong concentration and time responses. The implications of these results for the development of new nephrotoxicity models using donor derived iPSC will be highlighted.

III-8-609

Predictive toxicity testing in a microphysiological model of the human kidney proximal tubule

Edward Kelly
University of Washington, Seattle, WA, United States
edkelly@uw.edu

The kidney proximal tubule is the primary site of drug-induced nephrotoxicity. I will describe the development of a 3-dimensional flow-directed proximal tubule microphysiological system (MPS). The kidney MPS recapitulates the synthetic, metabolic and transport activities of kidney proximal tubule cells. This MPS is as an ideal platform for *ex vivo* modeling of nephrotoxicity. Towards this goal, we have evaluated nephrotoxicity in response to challenge with multiple toxicants, including the heavy metal pollutant cadmium, antisense oligonucleotides, the antibiotic polymyxin B and the Chinese herbal product aristolochic acid. We believe that MPS technologies will have major impacts on predictive toxicity testing and human risk assessment. Animal and *in vitro* systems do not always faithfully recapitulate drug and xenobiotic responses in the clinic or occupational/environmental exposures, respectively. MPS technologies will refine safety assessment and reduce our need for surrogate animal testing. An ultimate goal is to create integrated human MPS organ systems that could replace animal models.

III-8-717

Development of a microfluidic model of the human proximal tubule and glomerulus

Gretchen Mahler
Department of Biomedical Engineering, SUNY Binghamton, Binghamton, NY, United States
gmahler@binghamton.edu

Static cell cultures and animal models often do not accurately represent human renal physiology for drug toxicity studies. Dynamic, physiologically-based systems may better predict human response to therapeutics. Our group has developed a microfluidic device that incorporates physiologically relevant cell populations, shear stress, proximal tubule reabsorption, and glomerular filtration. Within this device human renal proximal tubule cells display increased tight junction formation, polarization, cytoskeletal reorganization, and active transport protein expression. Glomerular filtration is modeled with a 30-nm pore size polyethersulferone membrane seeded with human umbilical vein endothelial cells and conditionally immortalized human podocytes. The *in vitro* glomerulus is able to filter bovine serum albumin from culture medium at physiological flow rates. Renal disease states such as hypertension, diabetes mellitus, and nephrolithiasis have been recreated with the system. This device allows for a close approximation of the *in vivo* renal environment and provides an accurate, low-cost platform for testing new drug candidates.

III-8-723

Dynamic and kinetic effects of cyclosporine A in long term repeat dose exposures in an *in vitro* human renal model

Anja Wilmes[1], Alice Limonciel[1], Giada Carta[1], Thomas Ringer[2], Simone Van Breda[3], Jos Kleinjans[3] and Paul Jennings[1]

[1]Dept of Physiology and Medical Physics, Medical University of Innsbruck, Innsbruck, Austria; [2]Austrian Drug Screening Institute, Innsbruck, Austria; [3]Dept of Toxicogenomics, Maastricht University, Maastricht, The Netherlands

anja.wilmes@i-med.ac.at

Due to the role the kidney plays in elimination, it is exposed to a wide variety of xenobiotics, many of which have the potential to cause toxicity. Damage to the nephron is a concern as there is no *de novo* nephrogenesis and renal toxicity may accelerate the onset of chronic kidney disease. Hence the development of models to predict renal safety is of major importance. Here, we employed a human renal proximal tubular cell line together with biokinetics and omics to model compound exposure with potential toxicity. RPTEC/TERT1 cells were treated with the nephrotoxin cyclosporine A in a prolonged repeat dose exposure, using an extensive washout and recovery period followed by a second repeat dose treatment to investigate toxicity, recovery and a potential cellular memory effects. The time course of dynamic effects was integrated with the omic (metabolomics, transcriptomics, epigenetics) effects. The results and implications are discussed.

Session III-9: Update on Models for Genotoxicity and Cancer

Co-chairs
Richard Paules, NIEHS, NIH, Research Triangle Park, NC, United States
Jos Kleinjans, Maastricht University, Maastricht, The Netherlands

III-9-802

In vitro insights into chemical carcinogenesis

Nicole Kleinstreuer

NICEATM, Research Triangle Park, NC, United States
nicole.kleinstreuer@nih.gov

The evolution in toxicology testing to high-throughput screening (HTS) assays provides rapid, cost-effective, broad biological coverage on thousands of chemicals against hundreds of human cellular and molecular targets. Assays from the Tox21 and ToxCast HTS programs have been mapped to gene and protein level targets, which have in turn been mapped to mechanistic pathways including cancer hallmarks and characteristics of carcinogens. Probabilistic models such as Bayesian networks can incorporate this data into biologically-based frameworks and provide chemical-specific predictions based on cancer-relevant *in vitro* bioactivity profiles. We investigate Bayesian networks trained on data from known carcinogens, using both naïve priors and informative priors based on systematic literature review results. The goal of this model is to provide probabilities that particular chemical exposures would result in carcinogenesis or in a biological environment favorable for tumor development.

III-9-819

High-throughput approaches for characterizing genotoxicity and carcinogenic potential: Bridging classic and high-throughput approaches

Richard S. Paules, Jui-Hua Hsieh, Stephanie Smith-Roe and Kristine L. Witt

DNTP, NIEHS, NIH, Research Triangle Park, NC, United States
paules@niehs.nih.gov

The US Tox21 collaborative High-Throughput Screening (HTS) program has tested > 10K compounds in cell-based assays focused on nuclear receptor and stress response pathway signaling. Five assays detect activation of DNA damage responses or the requirement of DNA repair for cell survival. Each assay identified a nearly unique set of actives, with only modest overlap among actives. Combining results across assays increased sensitivity, and integrating actives among assays increased the positive predictive value for genotoxicity, allowing prioritization of compounds for additional testing using orthogonal approaches to clarify HTS findings. These include traditional Ames and *in vitro* micronucleus assays as well as a HT CometChip assay and a multiplexed flow cytometric assay to discriminate clastogens, aneugens, and non-genotoxicants. In addition, we are employing HT transcriptomic approaches to characterize chemical effects on biological processes, including DNA damage responses.

III-9-815

Novel data analysis approaches towards toxicogenomics-based evaluation of chemical carcinogenicity *in vitro*

Jos Kleinjans

Maastricht University, Maastricht, The Netherlands
j.kleinjans@maastrichtuniversity.nl

Life sciences and health research has become a data-driven discipline or information science. Research in the field of toxicogenomics increasingly relies on the use of and integration of heterogeneous and multimodal data sets covering the chemical, biological and pathological space, and by doing so is drawing from vast quantities of data. If these challenges can be adequately managed toxicogenomics will enable better predicting human toxicity and deepen our understanding of toxic mechanisms-of-action. Data analysis approaches for pattern recognition within and across toxicogenomics data sets will facilitate meeting with these challenges. Here, results from various pattern recognition methods for performing dose-over-time integration, identifying persistent feed forward loops, evaluating read across and transnationally analyzing *in vitro* data, applied to toxicogenomics data sets developed for the purpose of predicting genotoxicity and carcinogenicity *in vivo*, will be presented.

III-9-227

Predictivity and transferability of EpiSkin™ 3D Micronucleus Assay

Ying Yang[1], Nan Li[2], Lizao Chen[2], Qing Li[1], Xin Chen[1], Caichun Ke[1], Yanfeng Liu[2], Lingyan Zhong[2], Brigitte Faquet[3], Gladys Ouedraogo[3], Nathalie Alépée[3], José Cotóvio[3] and Alice Cai[2]

[1]Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, People's Republic of China; [2]L'Oréal Research & Innovation China, Shanghai, People's Republic of China; [3]L'Oréal Research & Innovation France, Aulnay sous Bois, France
yang99063@126.com

The EU regulation on Cosmetics prohibits the use of animals in genotoxicity testing since March 2009 for ingredients used in cosmetics, to address the poor specificity of current 2D *in vitro* genotoxicity tests, a 3D *in vitro* genotoxicity test method – EpiSkin™ Micronucleus Assay was successfully established in L'Oréal R&I China laboratory.

To further assess the transferability and reproducibility of EpiSkin™ Micronucleus Assay, following the technical training provided by L'Oréal R&I China, the test method was successfully transferred to Guangdong CDC laboratory with 6 chemicals, identical results were obtained in both laboratories.

These preliminary results appear promising as *in vitro* assay for assessing genotoxicity, allows further external and multi-laboratory test transfer and validation in China. The availability and validity of the EpiSkin™ Micronucleus Assay are of great significance for extending the applications of non-animal alternative testing methods in China.

III-9-839

The HESI TGx-28.65 genomic biomarker to detect DNA damage-inducing agents: Development and qualification status

Jiri Aubrecht

Safety Biomarkers, Pfizer, Groton, CT, United States
Jiri.Aubrecht@pfizer.com

Development and formal qualification of biomarkers as Drug Development tools that are applicable for decision making by regulatory agencies is a complex scientific endeavor. It requires a close collaboration among scientists from academia, industry and regulatory agencies. Since early 2000 and under the auspices of Health and Environmental Science Institute's genomic consortium, we have been pursuing the development and qualification of a genomic biomarker TGx-28.65. The goal of the qualification efforts is to implement TGx-28.65 as a tool for assessing oncogenic risk for drug candidates and environmental chemicals. The presentation will discuss the biomarker qualification as an iterative process that requires close collaboration among all stakeholders.

Session III-10: Models Used for Ecology and Ecotoxicology

Co-Chairs
Marc Leonard, L'Oréal Research & Innovation, Aulnay sous Bois, France
Dan Villeneuve, US EPA Mid-Continent Ecology Division, Duluth, MN, United States

III-10-781

Mechanisms of metal olfactory neurotoxicity and cellular protection in fish

Evan Gallagher[1] and Chase Williams[2]

[1]Environmental and Occupational Health Sciences, Gallagher, Seattle, WA, United States; [2]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States
evang3@u.washington.edu

Fish rely on olfaction to maintain neurobehaviors such as predator detection and avoidance, prey selection, social behavior, imprinting, and homing. However, exposure to trace metals such as cadmium (Cd) and copper (Cu) can disrupt these behaviors that are essential for survival. We have shown that exposure to low environmental levels of Cd can cause long-lasting deficits to neurobehavior in salmon which impacts ciliated olfactory sensory neurons (OSNs), impairs olfactory neurogenesis and elevates oxidative stress in the peripheral olfactory system. By contrast, coho maintain an active olfactory antioxidant defense system that can protect against metal olfactory injury. These antioxidant defenses include a multi-gene glutathione S-transferase (GST) system, metallthionein (*mt*), heme oxygenase 1 (*hmox1*), and two nuclear factor, erythroid 2-like 2 (*nfe2l2* or *nrf2*) paralogs which activate the transcription of a broad array of protective antioxidant genes in other species. Promoter flanking sequences were isolated from five of these genes that we hypothesize are important in mitigating metal injury to OSNs, including peroxiredoxin 1 (*prdx1*), glutamate-cysteine ligase (*gclc*), *hmox1*, and the GSTs *pi* and *rho* (*gstp*, and *gstr*), and that were examined for their potential for activation by nrf2. All antioxidant genes except *gstr* had a functional antioxidant response element (ARE) that fit the standard mammalian-derived canonical sequence that was activated by Nrf2. Ongoing studies in zebrafish, including Nrf2 transgenics, will enable us to better understand the role of fish antioxidant responses to metal olfactory injury. Our studies using two model species shed light on the cellular mechanisms, defenses, and targets of metal olfactory injury that can occur in fish exposed to pollutants.

Supported by NIEHS Superfund P42-04696.

III-10-582

The fish embryo model, update and challenges for its potential regulatory applications

Marc Leonard

Environmental Research Department, L'Oréal, Research & Innovation, Aulnay sous Bois, France
mleonard@rd.loreal.com

Fish are key aquatic organisms, but fall into the scope of several international regulations for the protection of animals used for scientific purposes. As a consequence, replacing animal testing for the environmental safety assessment of chemicals and effluents faces challenges when addressing issues such as fish long term toxicity, environmental endocrine modulation and bioaccumulation. In compliance with international animal welfare regulations, the fish embryo model appears as an ethically acceptable predictive model of acute fish toxicity. But still, adjustments are requested for regulatory acceptance. Developments are ongoing to optimize its prediction capacity even to more chronic toxicity endpoints. In addition, the model shows promises in the field of human toxicology screening in particular for developmental toxicity assessment. These recent progresses will be presented.

III-10-834

Evolution of the adverse outcome pathway (AOP) framework to support the use of new approach methodologies in regulatory decision-making

Daniel L. Villeneuve

Mid-Continent Ecology Division, US EPA, Duluth, MN, United States
villeneuve.dan@epa.gov

The adverse outcome pathway (AOP) framework has received considerable attention as a means to support greater use of new approach methodologies (e.g., high throughput and non-animal testing) for regulatory decision-making. Since it was first presented at the 8th World Congress in 2011, the framework has evolved considerably. Five core principles of AOP development were defined and guidance on how to document and disseminate AOPs via an internationally-harmonized knowledge-base (aopwiki.org) was provided. To date, over 200 AOPs have been entered and a systematic and transparent review process, has been implemented. With a critical mass of AOPs entered into the knowledgebase, application of AOP networks is being explored. Examples of the use of AOPs for quantitative effects prediction have also emerged. This presentation reviews the evolution and state of the science of the AOP framework.

The contents of this abstract neither constitute, nor necessarily reflect, U.S. EPA policy.

III-10-589

Novel reporter-gene *in vitro* assays in aquatic toxicology

Sebastian Lungu-Mitea, Agneta Oskarsson and Johan Lundqvist

Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden
sebastian.lungu@slu.se

Developing *in vitro* methods for aquatic toxicity is highly prioritized since most risk assessment relies on *in vivo* studies. We have established an *in vitro* method to study oxidative stress in fish. Transfection efficiency of commercially available transfection agents were tested in the *D. rerio* cell lines ZFL, ZF4, and Pac2. The most efficient reagent for each cell line was selected for further experiments, where cells were transfected with an oxidative stress responsive luciferase plasmid. The model was tested using known oxidative stress inducing chemicals (*tert*-butylhydroquinone, hydrogen peroxide, and sulforaphane). Of the investigated cell lines, ZF4 showed the highest sensitivity to known inducers and was prioritized for further experiments. The model was used to study oxidative stress potential of a panel of pesticides (deltamethrin, metazachlor, and others), of which most showed dose-response relationships with induction folds of up to 15 in the highest concentrations.

III-10-57

Behavioral screening of the LOPAC^{1280} library in zebrafish embryos

Sara Vliet[1,2], Trina Ho[1] and David Volz[1]

[1]Environmental Sciences, University of California Riverside, Riverside, CA, United States; [2]Environmental Toxicology Graduate Program, University of California Riverside, Riverside, CA, United States
svlie001@ucr.edu

Zebrafish spontaneous activity represents an early form of zebrafish motor activity and offers a potential readout for the identification of neuroactive compounds. However, despite widespread use, the predictive power of assays using spontaneous activity remain unclear. Using a high-content screening assay, we screened a library of well-characterized compounds (LOPAC^{1280}) to identify targets detected using spontaneous activity as a readout. Results revealed that (1) 8% of the library was biologically active at a limit concentration; (2) compounds affecting spontaneous activity spanned a broad array of targets; (3) 4% of compounds targeting neurotransmission impacted spontaneous activity; and (4) hypoactivity was observed for all hits, including those with opposing mechanisms of action for the same target. Therefore, while this assay identified potent neuroactive chemicals, our data suggests that spontaneous activity may lack the ability to discriminate compound modes of action.

III-10-209

Implementation of zebrafish ontologies for toxicology screening

Jon Hamm[1], Patricia Ceger[1], Rebecca Boyles[2], David Reif[3], Lyle Burgoon[4], Melissa Haendel[5], Nigel Walker[6] and Elizabeth Maull[6]

[1]ILS, Morrisville, NC, United States; [2]Bioinformatics, RTI International, Durham, NC, United States; [3]Biological Sciences, NCSU, Raleigh, NC, United States; [4]US Army ERDC, Research Triangle Park, NC, United States; [5]Medical Informatics and Clinical Epidemiology, OHSU, Portland, OR, United States; [6]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, United States
hammjt@niehs.nih.gov

NTP's Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program was created in part to facilitate development of standardized screening approaches for the use of zebrafish in hazard identification and risk assessment. Through interviews with zebrafish researchers, SEAZIT cataloged current zebrafish screening practices and determined that the data collected and analysis procedures are highly variable. An April 2017 workshop reviewed the state of the science for zebrafish screening data analytics. Workshop sessions covered standardized nomenclature (ontology) and the advantages and limitations of available ontological approaches and software. The workshop outlined best practices for data production and analysis and identified needs to advance the application of the zebrafish model in toxicology.

This effort was funded with U.S. Federal funds from the NIEHS/NIH/HHS under Contract HHSN27320140003C.

III-10-793

Daphnia pulex as a model system to examine long-term outcomes after multi-stressor exposures early in life

Julia M. Gohlke[1], Christopher Andrews[1], Kestral Miller[1], Jordan Nelson[2] and Tonia S. Schwartz[3]

[1]Virginia Tech, Blacksburg, VA, United States; [2]University of Alabama at Birmingham, Birmingham, AL, United States; [3]Auburn University, Auburn, AL, United States
jgohlke@vt.edu

Daphnia pulex is a well characterized ecotoxicology model system. Here we propose extension of its use for screening combinations of chemicals that may have subtle long-term effects at the organismal level difficult to detect in cell-based systems. We have characterized lifespan and reproduction parameters in this system after early life exposure to multiple stressors including low iron diet, low food ration, high maternal age, methylmercury, and selenium. Our results suggest interactive effects between maternal age and selenium and methylmercury and food ration. This system will be particularly useful for testing hypotheses of subtle and protracted responses to environmental cues, such that environmental variation experienced maternally or early in life shapes disease risk later in life.

Session III-11: Vaccine Development: Role of the 3Rs

Co-Chairs
Coenraad Henriksen, Institute for Translational Vaccinology, Bilthoven, The Netherlands
Richard Hill, Veterinary Biologics, IABS, Ames, IA, United States

III-11-360

Introducing VAC2VAC

Coenraad Henriksen[1], Catrina Stirling[2], Denis Lambrigts[3], Sylvie Ulrich[4], Hilde Depraetere[5], Odile Leroy[5], Judy MacArthur-Clark[6], Elisabeth Balks[7], Paul Stickings[8], Arjen Sloots[1], Anke Huckriede[9], Marlies Halder[10] and Joris Vandeputte[11]

[1]Institute for Translational Vaccinology, Bilthoven, The Netherlands; [2]Zoetis, London, United Kingdom; [3]GSK Vaccines, Wavre, Belgium; [4]Sanofi Pasteur, Lyon, France; [5]European Vaccine Initiative, Heidelberg, Germany; [6]JMC Consulting, Penarth, United Kingdom; [7]Paul-Ehrlich Institute, Langen, Germany; [8]National Institute for Biological Standards and Controls, Potters Bar, United Kingdom; [9]University Medical Center Groningen, Groningen, The Netherlands; [10]ECVAM-JRC, Ispra, Italy; [11]IABS, Geneva, Switzerland
Coenraad.Hendriksen@intravacc.nl

VAC2VAC is a novel and ambitious project under the EU Innovative Medicines Initiative (IMI2) that brings together a unique One Health consortium of human and animal health pharmaceutical companies, academia, translational research organisations, medicines control laboratories and regulatory bodies. Over 5 years, the project aims to develop new non-animal methods that will allow acceptance of the "consistency approach" for human and veterinary vaccines. Many current inactivated vaccines use animals (laboratory and target species) as part of routine batch quality control for potency and safety testing. While desirable for ethical and scientific reasons, many challenges exist when moving to animal free test systems. The project aims to promote global understanding and acceptance of these new non-animal methods to facilitate international harmonisation and improved vaccine availability globally. The presentation will outline the project in detail and discuss some early results.

III-11-284

Development of recombinant human diphtheria antitoxin

Jeffrey Brown[1], Esther Wenzel[2], Michael Hust[2] and Stefan Dübel[2]

[1]PETA International Science Consortium Ltd, London, United Kingdom; [2]Institut für Biochemie, Biotechnologie und Bioinformatik Abteilung Biotechnologie, Technische Universität Braunschweig, Braunschweig, Germany
jeffreyb@piscltd.org.uk

Diphtheria antitoxin (DAT) is a life-saving drug, but the way it and other antitoxins are manufactured hasn't changed in more than 100 years. As with most therapeutic antitoxins, DAT is produced from the serum of equines who have been hyperimmunised by repeated toxin injections. In addition to documented animal welfare problems at facilities producing equine serum, public health authorities have noted a global shortage of equine DAT and called for the development of alternative products. As a first step toward developing a non-animal replacement for equine DAT, the PETA International Science Consortium Ltd. is funding the development of human monoclonal antibodies against diphtheria toxin (DT) that can be produced in cell culture. This project has produced 127 unique DT-binding antibodies currently being evaluated for DT neutralization activity and other characteristics. Two of the first twelve antibodies evaluated neutralized DT in cell-based assays.

III-11-174

From preclinical research to marketed product, an integrated strategy to implement 3Rs by a vaccine manufacturer

Nolwenn Nougarede[1], Emmanuelle Coppens[1], Frédéric Moysan[1], Sue Nelson[2] and Sylvie Uhlrich[1]

[1]Sanofi Pasteur, Marcy l'Etoile, France; [2]Sanofi Pasteur, Toronto, Canada
nolwenn.nougarede@sanofi.com

The 3Rs principles have been established in 1959 and since then have been adopted widely and particularly in Europe with the European Directive 2010/63/EU. The vaccine industry has been committed to the 3Rs principles for several years for refining, reducing and replacing animal use in research, non-clinical safety and analytical testing. Whereas animal testing has been successfully removed from lot release testing of well-characterized human vaccines, laboratory animals continue to be used for safety and potency quality control testing for established vaccines as well as for preclinical research for new vaccine development.

The presentation will briefly review the use of laboratory animals in human vaccines research and testing and will describe Sanofi Pasteur's integrated program for implementing 3Rs principles in R&D and Industrial Affairs processes. It will highlight the barriers and the opportunities encountered when implementing 3Rs principles, as well as ongoing efforts that include external collaborations with other industries, public organizations and Health Authorities for the acceptance of alternative methods.

III-11-785

Tetanus and diphtheria combined vaccine *in vitro* potency analysis: Replacement of animal testing by ToBI test

Patricia Carneiro, Eliane Rosa, Giulia Preto, Anderson Silva, Ana Carla Locatelli, Juliana Galvão da Silva, Karina Scaramuzzi Tatiane Canhamero, Amanda Oliveira, Matheus Queiroz and Eliana Marengo

Butantan Institute, São Paulo, Brazil
patricia.carneiro@butantan.gov.br

Most of release tests described in compendial literature indicates animal testing for biologicals. As part of National Network of Alternative Methods in Brazil (ReNaMA), Butantan Institute is engaged with 3Rs approach in order to implement it in the QC labs. Our group aims to develop and validate alternative methods for release test – antitoxins and antivenoms sera, tetanus and diphtheria combined vaccines, and also for pyrogen detection. Here, it is shown the applicability of the ToBI test after its validation to estimate the potency not only the tetanus but also the diphtheria in combined vaccines. Our data met all acceptance criteria, as specificity, precision (< 14%), accuracy (80-120%), and *in vitro* and *in vivo* concordance showed strong correlation (R = 0.98) for both D and T components. The high relevance of these data for replacement of animal testing strongly impacts the routine of the Quality Control, turning the analyses faster, accurate and cheaper and, more importantly, saving the lives of hundreds of animals per year.

III-11-814

"3Rs" in action: A veterinary perspective

Richard Hill

Veterinary Biologics, IABS, Ames, IA, United States
arhill@iastate.edu

While "host" animal studies for biologics development has been practiced for centuries, the use of laboratory animals as surrogates in biologics research/development and production testing eventually became the norm or standard. However, even with humane considerations, it is clear that between the total numbers of animals used worldwide, the distress involved with many of the tests, and the rapid growth of the numbers and types of biologics that are currently available to consumers, action is needed to further the humane considerations for these types in tests. In 1959, "The Three Rs" (Replacement, Reduction and Refinement) was introduced as a means to achieve industry-wide goals on animal use. Although challenges remain, considerable progress has been made towards the "3R" initiative and this presentation will highlight some of the key successes that have occurred in the veterinary biologics field over time.

Reference

Brown, F., Hendriksen, C. and Sesardic, D. (eds.) (1999). Alternatives to Animals in the Development and Control of Biological Products for Human and Veterinary Use. London, United Kingdom, September 24-26, 1998. Proceedings. *Dev Biol Stand 101*, 1-335.

III-11-188

Benefits of the monocyte activation test in vaccine development and batch release

Ingo Spreitzer

Section 1/3 Microbial Safety, Paul-Ehrlich-Institut, Langen, Germany
ingo.spreitzer@pei.de

The Test for contaminating Pyrogens (fever inducers) is crucial in safety testing of pharmaceuticals. Classically performed by the Rabbit pyrogen test (RPT), today most drugs are tested for Endotoxin by the Bacterial Endotoxin test (BET). The BET gives an assumption about the Endotoxin content, but not about the biological impact (pyrogenicity) of the sample (Huang et al., 2009; Brandenburg et al., 2009). Different Endotoxins exhibit distinct biochemical and biological properties (Dehus et al., 2006; Greisman and Hornick, 1969; Bache et al., 2012). The use of modified LPS-structures (e.g. MPL), outer membrane vesicles (OMV) or Non-LPS adjuvants (e.g. Flagellin-derived) in vaccine formulations questions the use of the BET as release test (Gerke et al., 2015; Vipond et al., 2016). The intrinsic pro-inflammatory action of vaccines should be monitored by predictive and comprehensive tests (Vipond et al., 2016; Carlin and Viitanen, 2003, 2005).

Monocyte activation tests (MAT) have been developed as a replacement for the RPT, and are compendial in Europe since 2010. The use of human monocytes integrating the signals of various receptors is a state of the art approach for modern pyrogen testing (Spreitzer, 2017). MAT has been employed successfully for the batch release of modern OMV-based vaccines.

The MAT is a reliable pyrogen test for vaccine release and research.

References

Bache, C. et al. (2012). Bordetella pertussis toxin does not induce the release of pro-inflammatory cytokines in human whole blood. *Med Microbiol Immunol 201*, 327-335.

Brandenburg, K., Howe, J., Gutsman, T. and Garidel, P. (2009). The expression of endotoxic activity in the Limulus test as compared to cytokine production in immune cells. *Curr Med Chem 16*, 2653-2660.

Carlin, G. and Viitanen, E. (2003). In vitro pyrogenicity of a multivalent vaccine: Infanrix. *Pharmeuropa 15*, 418-423.

Carlin, G. and Viitanen, E. (2005). In vitro pyrogenicity of the diphtheria, tetanus and acellular pertussis components of a trivalent vaccine. *Vaccine 23*, 3709-3715.

Dehus, O., Hartung, T. and Hermann, C. (2006). Endotoxin evaluation of eleven lipopolysaccharides by whole blood assay does not always correlate with Limulus amebocyte lysate assay. *J Endotoxin Res 12*, 171-180.

Gerke, C. et al. (2015). Production of a Shigella sonnei vaccine based on generalized modules for membrane antigens (GMMA), 1790GAHB. *PLoS One 10*, e0134478.

Greisman, S. E. and Hornick, R. B. (1969). Comparative pyrogenic reactivity of rabbit and man to bacterial endotoxin. *Proc Soc Exp Biol Med 131*, 1154-1158.

Huang, L. Y., Dumontelle, J. L., Zolodz, M. et al. (2009). Use of toll-like receptor assays to detect and identify microbial contaminants in biological products. *J Clin Microbiol 47*, 3427-3434.

Spreitzer, I. (2017). Pyrogen testing in the 21st century. *BioPharma Asia 6*, 24-29.

Vipond, C., Findlay, L., Feavers, I. and Care, R. (2016). Limitations of the rabbit pyrogen test for assessing meningococcal OMV based vaccines. *ALTEX 33*, 47-53.

III-11-61

Human rabies vaccine potency testing; the test for G-protein: Report of the pre-collaborative study and future strategies

Jean Michel Chapsal[1] *and Eriko Terao*[2]

[1]Independant expert, Charnay, France; [2]Dept of Biological Standardisation, OMCL Network & HealthCare (DBO), EDQM, Strasbourg, France
jmchapsal@gmail.com

Numerous studies have shown that human immunization with the native immunogenic form of the rabies glycoprotein G results in the production of neutralizing antibodies and protection against lethal challenge. In human rabies vaccines manufacturing, antigen quantification is used at final bulk stage, allowing definition of the vaccine final antigen content. Official release control of Human rabies vaccines relies upon an *in vivo* potency test in mice, the NIH test (Chabaud-Riou et al., 2017). This test is based on immunisation-intracranial virus challenge model and is using a large number of mice. An EPAA project meeting in 2012 focused on gaps in technical knowledge, validation of *in vitro* G antigen quantification methods and finally proposed solutions for the replacement of the NIH test by one of these *in vitro* methods. Regulators and manufacturers stressed that the NIH test should be replaced and emphasized that the current *in vivo* assay should not be used for correlation with the *in vitro* methods, since it is highly variable and therefore a concordance strategy should be followed. The ELISA methods for the G antigen quantification under development should be able to discriminate between potent and sub-potent batches (agreement study). An International Working Group including regulators, rabies science specialists and manufacturers was formed to coordinate a more harmonised approach of the alternative assays through the acquisition and distribution of a common set of rabies vaccines. A protocol was established to allow preliminary comparison of different ELISA methods using potent and sub potent human rabies lots. Results from these studies were presented mid 2015 at an EPAA group workshop. They have indicated the good agreement of the ELISA methods with the NIH test (Chabaud-Riou et al., 2017). An ELISA method was selected (Morgeaux et al., 2017; Seligmann, 1973) for its ability to discriminate potent from sub potent lots but also to detect efficaciously the two main strains used in rabies vaccines manufacturing (PM and Flury LEP). The group decided then to evaluate further this ELISA method for its ability to recognise different rabies strains used for vaccine manufacturing around the world. Results of these studies have form the basis for the launch in 2017 of an EDQM collaborative study, Biological Standardization Program 148. This BSP study should conduct to the replacement of the *in vivo* NIH test by an *in vitro* method as official release potency test of human rabies vaccines around the world

References

Chabaud-Riou, M., Moreno, N., Guinchard, F. et al. (2017). G-protein based ELISA as a potency test for rabies vaccines. *Biologicals 46*, 124-129.

Morgeaux, S., Poirier, B., Ragan, C. I. et al. (2017). Replacement of in vivo human rabies vaccine potency testing by in vitro glycoprotein quantification using ELISA – Results of an international collaborative study. *Vaccine 40*, 369-381.

Seligmann, E. B. (1973). The NIH test for potency. In M. M. Kaplan and H. Koprowski (eds.), *Laboratory Techniques in Rabies* (279-286). 3rd edition. Geneva: WHO.

III-11-383

cAMP reporter cell lines to replace the histamine sensitization test

Marieke Hoonakker[1]*, Lisa M. Verhagen*[1]*, Larissa van der Maasa*[1]*, Arjen Sloots*[1] *and Coenraad C. F. Hendriksen*[1,2]

[1]Intravacc, Bilthoven, The Netherlands; [2]Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
Marieke.Hoonakker@intravacc.nl

Pertussis toxin (PTx) is one of the virulence proteins of *Bordetella pertussis*. Its detoxified form, pertussis toxoid (PTd), contributes to protection and is the major antigen of acellular pertussis (aP) vaccines. Because of the risk of residual toxicity and reversion to toxicity, the *in vivo* Histamine Sensitization test (HIST) in mice is regulatory required to examine possible toxin content of all aP vaccines. In the last decades, the intrinsic limitations of this test – including a lack of mechanistic understanding, technical handicaps and animal welfare concerns – have pushed the search for alternative methods.

As the cellular effects of PTx primarily interfere with intracellular pathways that involve cAMP, we generated reporter cell lines that stably express a reporter construct responsive to changes in intracellular cAMP levels. Two reporter cell lines were developed (CHO-CRE and A10-CRE) that enabled the detection of PTx in a concentration-dependent manner. The CHO-CRE cell line also detected PTx in the context of an aP containing multivalent vaccine adjuvanted with aluminium phosphate, with a sensitivity equal to the HIST. A10-CRE cells were less sensitive. In addition, the CHO-CRE reporter cell line might allow assessment of the cellular effects caused by reversion of PTd to PTx.

These results demonstrate that the CHO-CRE reporter cell line enables simple, quantitative and concentration-dependent detection of PTx, features that are not offered by the CHO cell clustering assay, another cellular alternative to the HIST. Therefore, the CHO-CRE reporter cells are a promising *in vitro* method to replace the suboptimal *in vivo* test.

Session III-12: Applications of the 3Rs for Evaluating Medical Devices

Co-Chairs
Sebastian Hoffmann, seh consulting + services, Paderborn, Germany
Ronald Brown, US Food and Drug Administration, Silver Spring, MD, United States

III-12-797

An introduction to international biocompatibility testing of medical devices

Sebastian Hoffmann[1] and Michael F. Wolf[2]

[1]seh consulting + services, Paderborn, Germany; [2]Medtronic Inc., Minneapolis, MN, United States
sebastian.hoffmann@seh-cs.com

Traditionally, medical device biocompatibility testing has been dominated by *in vivo* tests. With recent advancements in the development of non-animal test methods, various efforts are under way to evaluate and validate *in vitro* methods for testing of biomaterials. International biological safety testing requirements for medical devices will be presented with a focus on the most relevant toxicological endpoints. Round robin studies to validate the use of reconstituted human epidermis models for testing medical device extracts, to compare various protocols for assessing the hemolysis potential of medical devices and to optimise and evaluate an *in vitro* approach for thrombogenicity will be briefly introduced.

As the range of medical device materials and applications expands, it can be expected that the development and application of *in vitro* assays will contribute to the challenge of supporting new therapies, improving medical materials, and advancing patient care with meaningful and clinically relevant preclinical safety tests.

III-12-835

Use of computational approaches to assess the toxicity of compounds extracted from medical device materials

Ronald Brown

US Food and Drug Administration, Silver Spring, MD, United States
Ronald.Brown@fda.hhs.gov

The FDA Center for Devices and Radiological Health (CDRH) recently issued a guidance document that provides recommendations for the biocompatibility assessment of medical devices and selection of appropriate biological endpoints for the safety evaluation. This new CDRH guidance endorses the principle stated in the ISO 10993-1:2009 standard to minimize the "number and exposure of test animals by giving preference to chemical constituent testing and *in vitro* models, in situations where these methods yield equally relevant information to that obtained from *in vivo* models." Chemical constituent testing, in conjunction with toxicological risk assessment, is being increasing used as an alternative to the use of animal models to assess the biological safety of medical devices. As part of this evaluation, toxicity data are needed to derive exposure limits for each of the chemical substances extracted or leached from polymeric materials; however, experimentally derived toxicity data are not available for many of these compounds. In the absence of these data, computational approaches such as structure-activity relationship (SAR) models and Read Across methods can be used to estimate the toxic potential of the substances released from device materials. This talk with provide practical guidance on the use of SAR models and Read Across approaches to estimate the toxicity of extractable and leachable substances and will provide case studies illustrating how these approaches can be used for the toxicological safety evaluation of these substances.

III-12-783

Round robin study to evaluate the Reconstructed human Epidermis (RhE) model as an *in vitro* skin irritation test for medical devices

Kelly Coleman[1], Wim De Jong[2], Sebastian Hoffmann[3], Michelle Lee[4], Helena Kandarova[5], Silvia Letasiova[5], Liset De La Fonteyne[2], Christian Pellevoisin[6], Carine Tornier[6], Cristelle Videau[6], Yuji Haishima[7], Austin Zdawczyk[8], Audrey Turley[4], Jamin Willoughby[9], Michael Bachelor[10] and Beau Rollins[11]

[1]Medtronic plc, Minneapolis, MN, United States; [2]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; [3]seh consulting + services, Paderborn, Germany; [4]Nelson Laboratories, Inc., Salt Lake City, UT, United States; [5]MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia; [6]EpiSkin Academy, Lyon, France; [7]Division of Medical Devices, National Institute of Health Sciences, Tokyo, Japan; [8]NAMSA, Northwood, OH, United States; [9]Cyprotex US LCC, Kalamazoo, MI, United States; [10]MatTek Corporation, Ashland, MA, United States; [11]Arthrex, Inc., Naples, FL, United States
kelly.p.coleman@medtronic.com

Assessment of dermal irritation is an essential *in vivo* component of medical device safety evaluation. In 2016, an international round robin validation study was conducted to assess two RhE models as alternatives: EpiDerm (MatTek, Inc.) and SkinEthic RHE (EpiSkin, SA). Blinded samples of irritant polymers and controls were extracted with sesame oil and saline by 19 laboratories. Tissues were dosed with extracts then incubated at 37°C with 5% CO_2. After incubation and rinsing with PBS, cell viability was determined by MTT. Cell viability reduction greater than 50% was indicative of skin irritation. Both types of tissues were able to correctly identify virtually all of the irritant polymer samples. Our results indicate that RhE tissue models can detect the presence of strong skin irritants at low levels in dilute medical device polymer extracts. Therefore, these models may be suitable replacements for the rabbit skin irritation test to evaluate medical device biocompatibility.

III-12-256

Simulating osteoarthritis *in vitro* with human scaffold-free 3D cartilage transplants

Annemarie Lang[1,2,3], Marie-Christin Weber[1], Igor Ponomarev[4], Moritz Pfeiffenberger[1,3,5], Timo Gaber[1,3] and Frank Buttgereit[1,3]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Berlin- Brandenburg School of Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany; [3]German Rheumatism Research Centre, Berlin, Germany; [4]Research Center of Medical Technology and Biotechnology (fzmb GmbH), Bad Langensalza, Germany; [5]Dahlem Research School, Freie Universität Berlin, Berlin, Germany
annemarie.lang@charite.de

We aim to generate a feasible 3D *in vitro* osteoarthritis (OA) model that consists exclusively of the involved cells and their metabolic products. Based on the scaffold-free 3D cartilage transplant (SFCT) technology developed at the fzmb GmbH, we are able to establish single- and multi-component models. SFCTs were generated using human bone marrow derived mesenchymal stromal cells. To mimic the *in vivo* inflammatory environment of OA, SFCTs were treated with IL-1β and TNF-α for 3 weeks and compared to untreated controls. After stimulation of SFCTs, the inflammatory marker gene expressions and matrix degrading enzyme gene expressions were increased as compared to controls. The histological findings showed increased softening and wateriness of the tissue. A histological redistribution of Collagen I and II production could be demonstrated. Proteomics and mRNA analysis support the described results. The observed effects were reversible after 3 weeks of cytokine withdrawal.

III-12-257

Modelling the initial phase of fracture healing *in vitro*

Moritz Pfeiffenberger[1,2,3], Igor Ponomarev[4], Janika Bartsch[1], Alexandra Damerau[1,3], Frank Buttgereit[1,3], Timo Gaber[1,3] and Annemarie Lang[1,3,5]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Dahlem Research School, Freie Universität Berlin, Berlin, Germany; [3]German Rheumatism Research Centre, Berlin, Germany; [4]Research Center of Medical Technology and Biotechnology (fzmb GmbH), Bad Langensalza, Germany; [5]Berlin-Brandenburg School of Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany
moritz.pfeiffenberger@charite.de

Fracture healing dysfunctions occur in up to 10% of human patients. To provide a system to test new therapeutic approaches and to study underlying pathways, we aim at developing a valid 3D model to simulate the initial phase of fracture healing *in vitro*. To this end, we developed and characterized a human fracture hematoma 3D model consisting of a clot of human blood pre-mixed with mesenchymal stromal cells. The results demonstrate striking similarities when compared to *ex vivo* data from human patients with regard to cell composition and mRNA-expression pattern. The fracture hematoma model was embedded in 3D matrix-free bone-like constructs to mimic the bone epiphysis. The bone-like constructs, examined by qRT-PCR, histology, μCt and immunohistochemistry showed characteristics of endochondral ossification. The established 3D model enables us to study the early phase of fracture healing *in vitro*, and to test new therapeutic strategies and simulate different disease modes.

III-12-254

Development of an *in vitro* multi-component 3D arthritic joint model

Alexandra Damerau[1,2], Moritz Pfeiffenberger[1,2,3], Frank Buttgereit[1,2], Timo Gaber[1,2] and Annemarie Lang[1,2,4]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]German Rheumatism Research Centre, Berlin, Germany; [3]Dahlem Research School, Freie Universität Berlin, Berlin, Germany; [4]Berlin-Brandenburg School of Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany
alexandra.damerau@charite.de

The *in vitro* 3D joint model consists of an osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane. Human bone marrow derived mesenchymal stromal cells (hMSC) are used to develop the different 3D tissue components that are characterized in detail using histological, biochemical and molecular biological methods as well as *in vitro* μCT and scanning electron microscope. First results for single and co-cultivation are promising with respect to a successful colonization of hMSC on the used β-tricalcium phosphate particles (osteogenic component) and verification of the scaffold-free 3D chondrogenic structures. By combining the different components in a standard 96 well format, we aim to provide a high throughput system for preclinical drug testing as well as a valid *in vitro* 3D disease model to study with human material the immune mediated pathogenesis of arthritis.

Session III-13: Regulatory Issues Related to Organs on a Chip – Round Table

Chair
Suzanne C. Fitzpatrick, US Food and Drug Administration, Silver Spring, MD, United States

III-13-805

Advancing regulatory toxicology at FDA

Suzanne C. Fitzpatrick
US Food and Drug Administration, Silver Spring, MD, United States
Suzanne.fitzpatrick@fda.hhs.gov

Toxicology is transitioning from dependence on animal studies and apical endpoints towards more emphasis on mechanistic data to enhance the efficiency & effectiveness of chemical risk management. Collaborations between government researchers and regulators and outside stakeholders are important to ensure the most promising technologies are identified, developed, validated and integrated into the regulatory paradigm. The Food and Drug Administration (FDA) has formed a Senior Toxicologist Working Group to develop a framework for advancing predicative toxicology that emphasizes a "context of use" approach for developing confidence in new methodologies. The Center for Food Safety and Applied Nutrition (CFSAN) is the FDA lead for the partnership between FDA, EPA, NIEHS and NCATS that forms the "Tox 21 Community." This important collaboration ensures continued cooperation between these agencies to advance regulatory toxicology. One of the most successful partnerships for advancing regulatory science is the FDA DARPA NCATS initiative, started in 2011, to support the development of human microphysiological systems, or organ "chips." CFSAN has recently signed a Cooperative Research and Development Agreement to test in FDA laboratories the effectiveness of this technology to better understand the effects of medicines, disease-causing bacteria in foods, chemicals, and other potentially harmful materials on the human body.

Session III-14: High Content and 3D Organ Models

Co-Chairs
Maurice Whelan, Joint Research Center, European Commission, Ispra, Italy
Suzanne C. Fitzpatrick, US Food and Drug Administration, Silver Spring, MD, United States

III-14-135

Integrated multi-organ-on-chip platform

Y. Shrike Zhang
Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, United States
yszhang@mit.edu

We have developed a fully integrated multi-organ-on-a-chip platform consisting of both biomimetic human organoid models and auxiliary sensing units for continuous, *in situ* monitoring of organoid behaviors towards pharmaceutical compounds in an automated and uninterrupted manner. The platform was designed to be modular, consisting of a breadboard for microfluidic routing via built-in pneumatic valves, microbioreactors for hosting organoids, a physical sensing unit for measurement of biophysical parameters, and electrochemical sensing units for detection of soluble biomarkers secreted by the organoids. As such, we successfully achieved long-term monitoring of drug-induced organoid toxicity in a human liver-and-heart-on-a-chip platform insulted by acetaminophen for 5 days and a human liver-cancer-and-heart-on-a-chip platform challenged with doxorubicin for 24 h. We believe that our novel platform will provide a new method for integrating existing biomimetic organoid models with a potential to achieve large-scale automation in the drug screening process.

Reference

Zhang, Y. S., Aleman, J., Shin, S. R. et al. (2017). Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. *Proc Nat Acad Sci U S A 114*, E2293-E2302.

III-14-303

In vitro vasculogenesis to interconnect organoids in a multi-organ-chip platform

Juliane Hübner[1], Tobias Hasenberg[1], Katharina Schimek[1,2], Severin Mühleder[3], Sophie Bauer[1], Johanna Wachsmuth[2], Stefan Sieber[2], Wolfgang Holnthoner[3], Heinz Redl[3], Roland Lauster[2] and Uwe Marx[1]

[1]TissUse GmbH, Berlin, Germany; [2]Medical Biotechnology, Technische Universität Berlin, Berlin, Germany; [3]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

t.hasenberg@tissuse.com

The Multi-Organ-Chip is a microphysiological system developed to evaluate toxicity of drugs, cosmetics and alike. The system comprises compartments for the co-cultivation of human 3D tissue constructs. Organoids are physically separated, yet, interconnected through perfused microfluidics. Minute volumes of medium enable crosstalk. The organoid cultures are, however, not sufficiently vascularised to overcome limitations in size and complexity. A continuous endothelium, further, is crucial for physiological-like interactions, regulation and homeostasis within organoid (co-)cultures.

Three major aspects were addressed: (1) Implementing a near-physiological, pulsatile flow. It provides an *in vivo*-like shear stress regime. (2) Creating an endothelial lining within the chip's microfluidic system. The optimised flow promotes long-term vitality and the expression of typical endothelial markers. (3) Establishing capillary-like vessels as a direct route to the organoids. Fibrin hydrogels containing an endothelial/stromal cell co-culture enable the self-organised formation of microcapillaries and their connection to a bone marrow model.

III-14-596

A human heart-liver-skin microfluidic platform to assess systemic toxicity of compounds absorbed through the skin

Carlos A Carmona-Moran[1], Carlota Oleaga-Sancho[1], Andrea Caetano Das neves Lavado[1], Sandra Rothemund[1], Richard L. Bridges[1], Case Lorance[1], Christopher McAleer[1], Christopher J. Long[1], Jessica Langer[2], Anne Riu[3], Reine Note[3], Silvia Teissier[3], José Cotovio[3] and James J. Hickman[1]

[1]NanoScience Technology Center, University of Central Florida, Orlando, FL, United States; [2]Research and Innovation, L'Oréal, Clark, NJ, United States; [3]Research and Innovation, L'Oréal, Aulnay sous Bois, France

carlosar@ucf.edu

Assessing systemic toxicity from topically absorbed chemicals is crucial for development of new cosmetic ingredients. Current *in vitro* skin models can't predict the long-term systemic toxicity; animal models have limited predictivity and are banned for cosmetics since 2013. "Body-on-a-chip" platforms offer a relevant, physiological model for multi-organ interactions that can be used to test systemic responses. We have developed a novel human heart-liver-skin microfluidic chip linking cardiac, hepatic and reconstructed skin modules to evaluate a potential toxicity through topical exposure. The platform was used to evaluate the effects on hepatic and cardiac functions of 4 drugs such as diclofenac, acetaminophen, ketoconazole and hydrocortisone, applied to the skin. The organ modules maintained full functionality in serum-free medium, under flow, for 14 days. This *in vitro* model allows elucidation of chronic topical exposure to predict potential organ toxicity.

III-14-98

Establishment and characterization of a lung/liver organ-on-a-chip model

David Bovard, Arno Knorr, Antonin Sandoz, Karsta Luettich, Stefan Frentzel, Diego Marescotti, Keyur Trivedi, Emmanuel Guedj, Nikolai Ivanov and Julia Hoeng

PMI R&D, Philip Morris Products S.A., Neuchâtel, Switzerland

david.bovard@contracted.pmi.com

In order to be able to more accurately assess the toxicity of aerosols, we have developed a new multiorgan-on-a-chip system combining 3D organotypic lung tissues with HepaRG™ spheroids. After an extensive characterization of the tissues in single culture, we verified that the spheroids would metabolically act as a human liver equivalent. In addition to examining the expression and activity of selected P450 enzymes, we measured metabolite formation following exposure of the liver tissues to nicotine or NNK. We detected the major nicotine and NNK metabolites normally found in smokers, confirming the spheroids' metabolic capacity. Using our in-house multiorgan-on-a-chip, we then assessed the health status of both cultures individually and in co-culture. The results presented here provide an overview of our efforts to date.

III-14-97

Microphysiological human tissue models for biomedical applications

Marko Gröger[1], Martin Raasch[1], Anja Träger[2], Ulrich S. Schubert[2], Michael Bauer[3], Oliver Werz[4] and Alexander S. Mosig[1]

[1]Institute of Biochemistry II and Center for Sepsis Control and Care, University Hospital Jena, Jena, Germany; [2]Institute of Organic Chemistry and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Jena, Germany; [3]Department of Anesthesiology and Intensive Care, Center for Sepsis Control and Care, University Hospital Jena, Jena, Germany; [4]Institute of Pharmacy, Friedrich Schiller University Jena, Jena, Germany
marko.groeger@med.uni-jena.de

Microphysiological biochip based human organ models enter stage in biomedical research. These models already allow a precise adjustment of an organ-specific microenvironment to emulate physiological conditions or pathophysiological processes during disease progression *in vitro*. Based on the Multi Organ Tissue Flow (MOTiF) biochip we established scalable multilayered 3D tissue models reflecting essential structural aspects of *in vivo* organ morphology. Natural compounds as well as nanoparticles were tested in organ-on-chip models emulating the human vasculature, liver or tumor tissue. Here we could proof the superiority of organ-on-chip technology with its scalable cellular complexity and the advantage of tissue culture under physiological relevant conditions over conventional static cell cultures. Thus, these microphysiological models represent a valuable tool in translational research and drug screening as an alternative to animal experimentation.

References

Englert, C., Trützschler, A. K., Raasch, M. et al. (2016). Crossing the blood-brain barrier: Glutathione-conjugated poly(ethylene imine) for gene delivery. *J Contr Release 241*, 1-14.

Gröger, M., Rennert, K., Giszas, B. et al. (2016). Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model. *Sci Rep 6*, 21868.

Pergola, C., Schubert, K., Pace, S. et al. (2017). Modulation of actin dynamics as potential macrophage subtype-targeting anti-tumour strategy. *Sci Rep 7*, 41434.

Rennert, K., Steinborn, S., Gröger, M. et al. (2015). A microfluidically perfused three dimensional human liver model. *Biomaterials 71*, 119-131.

Rinkenauer, A. C., Press, A. T., Raasch, M. et al. (2015). Comparison of the uptake of methacrylate-based nanoparticles in static and dynamic in vitro systems as well as in vivo. *J Control Release 216*, 158-168.

III-14-506

Cross-laboratory testing of the human proximal tubule tissue chip

Courtney Sakolish[1], Cody Bell[1], Elijah J. Weber[2], Edward J. Kelly[2], Jonathan Himmelfarb[3], Roula Mouneimne[1], Terry Wade[4], Arum Han[5,6], Weihsueh Chiu[1] and Ivan Rusyn[1]

[1]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, United States; [2]Department of Pharmaceutics, University of Washington, Seattle, WA, United States; [3]Division of Nephrology, University of Washington, Kidney Research Institute, Seattle, WA, United States; [4]Geochemical and Environmental Research Group, Texas A&M University, College Station, TX, United States; [5]Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX, United States; [6]Department of Biomedical Engineering, Texas A&M University, College Station, TX, United States
csakolish@cvm.tamu.edu

Texas A&M University is conducting testing of microphysiological systems (MPSs) with the goal of evaluating the functionality, reproducibility, robustness and reliability of models representing an array of human organs and tissues. This center has engaged with the NIH tissue chip grantees, IQ consortium, experts in the pharmaceutical and chemical industry, government agencies, and academia to address validation. The first MPS evaluated was the proximal tubule, developed by the University of Washington. The functionality of this system has been validated along parameters previously published by the developer lab in three rounds of testing: non-specific binding, functionality (cell viability, protein and gene expression, ammoniagenesis, metabolism, and drug toxicity), and toxicological responses. These tests are carried out in parallel with static culturing in a true "fit for purpose" validation, addressing the much-needed external evaluation of MPSs against current "gold standards".

III-14-277

Pharmacokinetics of acetaminophen in a 2-Organ-Chip (2-OC) platform

Talita Marin[1], Silvana Rocco[1], Raphael Neto[2], Nathalia Indolfo[1], Alisson Cardoso[1] and Eduardo Pagani[1]

[1]LNBio/CNPEM, RENAMA, Campinas, Brazil; [2]LNBio/CNPEM, NMR Laboratory, Campinas, Brazil
talita.marin@lnbio.cnpem.br

Microfluidic systems are promising for developing better non-animal tests, but pharmacokinetic (PK) data in chips are missing. Acetaminophen (APAP) is 95% conjugated by the liver before renal excretion, making the liver the main organ for APAP metabolism. This study addressed the APAP PK properties in the 2-OC (by TissUse GmbH). A 2-OC was assembled with an intestinal barrier of CaCo2 + HT29 cells in one compartment with microfluidic communication with the other having liver spheroids of HepG2 + Stec Cell. The administration route was emulated by APAP placement on the intestinal barrier (oral) or in the media (intravenous). Media samples were collected and analyzed by HPLC/UV. Preliminary data show slower absorption through the CaCo2 + HT29 cells than observed in humans. This is expected due to CaCo2 thigh junctions. Main APAP hepatic metabolites have been found, showing HepG2 metabolic capability. These data show that 2-OC is able to emulate some PK functions and can help on system's optimization.

References

Gordon, S., Daneshian, M., Bouwstra, J. et al. (2015). Non-animal models of epithelial barriers (skin, intestine and lung) in research, industrial applications and regulatory toxicology. *ALTEX 32*, 327-378.

Marx, U., Andersson, T. B., Bahinski, A. et al. (2016). Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. *ALTEX 33*, 272-321.

Maschmeyer, I., Lorenz, A. K., Schimek, K. et al. (2015). A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip 15*, 2688-2699.

Rebelo, S. P., Dehne, E. M. and Brito, C. (2016). Validation of bioreactor and human-on-a-chip devices for chemical safety assessment. *Adv Exp Med Biol 856*, 299-316.

Session III-15: Considerations of Exposure Dose and Metabolism in 3Rs

Co-Chairs
John Wambaugh, US EPA, Research Triangle Park, NC, United States
Nynke Kramer, Utrecht University, Utrecht, The Netherlands

III-15-592

Exposure-based screening and priority-setting

John Wambaugh[1], Dustin Kapraun[2], Kristin Isaacs[3], Jon Sobus[3], Katherine Phillips[3] and Woodrow Setzer[1]

[1]National Center for Computational Toxicology, U.S. E.P.A., Research Triangle Park, NC, United States; [2]National Center for Environmental Assessment, U.S. E.P.A., Research Triangle Park, NC, United States; [3]National Exposure Research Laboratory, U.S. E.P.A., Research Triangle Park, NC, United States
wambaugh.john@epa.gov

The U.S. National Academy of Sciences report "Using 21st Century Science to Improve Risk-Related Evaluations" recognized that high-throughput screening (HTS) and exposure prediction tools are necessary to prioritize thousands of chemicals with the potential to pose human health risk. High-throughput models, utilizing machine learning tools, can now predict human exposure rates based upon chemical structure and use, thus filling critical gaps for thousands of compounds. These models can be calibrated to existing exposure monitoring data, allowing evaluation of their predictive ability and empirical assessment of their certainty. These tools provide real-world context for *in vitro* HTS efforts. In addition, new informatics tools, along with non-targeted analytical chemistry methods, allow surveillance of the environment to identify new candidates for HTS. Together, exposure prediction and surveillance allow HTS to be more timely and relevant to human health risks.

This abstract does not necessarily reflect U.S. EPA policy.

III-15-140

Developing a strategy to distinguish between the variability in biokinetics and toxicodynamics *in vitro*

Emma E. J. Kasteel, Bas J. Blaauboer and Nynke I. Kramer

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
e.kasteel@uu.nl

Adverse outcome pathways (AOPs) afford a straightforward, mechanistic insight into toxicological processes. As such, they are useful tools for developing an animal test-free toxicity testing strategy. We aim to develop *in vitro* methods to quantify AOPs and assess the human variability of toxicodynamic (TD) events herein. We identified key events (KEs) involved in the hepatic steatosis AOP from literature and setup a battery of *in vitro* assays testing for biomarkers in HepaRG cells. Amiodarone, fumonisin B1 and fipronil were exposed to the *in vitro* battery and their *in vitro* distribution as well as effect on KE biomarkers were measured over time. A biokinetic/toxicodynamic (BK/TD) model was established to simulate the concentration- and effect-time profiles for each biomarker. We provide a proof-of-principle approach to quantify an AOP and distinguish between BK and TD processes *in vitro*, which in the future can be used to identify human variation in BK and TD processes separately.

III-15-480

Incorporating metabolism into a tiered testing scheme based on the combination of *in vitro* and *in silico* approaches

Miyoung Yoon[1], Martin Phillips[1], Jeffrey Enders[1], Pergentino Balbuena-Venancio[1], Saad Haider[2], Chantel Nicholas[2] and Harvey Clewell[1]

[1]Biokinetics and Biosimulations, ScitoVation, LLC, Research Triangle Park, NC, United States; [2]ScitoVation, LLC, Research Triangle Park, NC, United States
myoon@scitovation.com

Human relevant *in vitro* assays and predictive computational tools have the promise for increasing efficiency of toxicity testing and its relevance to safety assessment. To increase confidence in *in vitro*-based safety decisions, metabolism needs to be considered in addition to the biological relevance of the *in vitro* models. Prediction of the biologically active entity (parent and/or metabolite) and its *in vivo* clearance is critical in interpreting *in vitro* concentration-effect relationships in the context of *in vivo* safe exposure. Current efforts to improve *in vitro* metabolism models based on advanced hepatocyte culture methods will be presented. Once the data for metabolic clearance and metabolite profiles are acquired for a broad range of chemicals, they can be used to improve *in silico* metabolism prediction tools. The advanced liver models can be used to streamline metabolism and toxicity testing in realistic exposure scenarios including repeated exposure. A workflow to incorporate metabolism into a tiered testing scheme will be discussed based on the combination of *in vitro* models of different biological complexity along with computational tools.

III-15-555

Use of human skin equivalent models for an integrated approach for physiologically-based toxicokinetic (PBTK) testing

Zee Upton[1], James Chun Yip Chan[2], John Common[1], Christabelle Si Mei Goh[1], Dorinda Yan Qin Kioh[2], Chris Soon Heng Tan[3], Hao Fan[4], Ee Chee Ren[5], Vachiranee Limviphuvadh[4], Sebastian Maurer-Stroh[4], Qasem Alramadan[6], Pär Nordlund[3], Anthony Teik Jin Goon[7], Keng-Hwee Chiam[4] and Eric Chun Yong Chan[2]

[1]Institute of Medical Biology, A*STAR, Singapore; [2]Pharmacy, National University of Singapore, Singapore; [3]Institute of Molecular and Cell Biology, A*STAR, Singapore; [4]Bioinformatics Institute, A*STAR, Singapore; [5]Singapore Immunology Network, A*STAR, Singapore; [6]Institute of Microelectronics, A*STAR, Singapore; [7]National Skin Centre, Singapore
zee.upton@imb.a-star.edu.sg

Allergic contact dermatitis (ACD) is a complex condition that affects 15-20% of the general population, resulting in loss of productivity and poor quality of life. Common chemical allergens are found in numerous consumer goods and include metals, fragrances, preservatives and dyes. The formally validated murine local lymph node assay (LLNA) has proven to be a reliable tool to detect and rank the sensitization potential of chemicals. Nonetheless, regulatory developments preclude the use of animal testing, while fundamental differences in murine versus human skin and immune biology necessitate the development of fit-for-purpose human *in vitro* methodologies to complement the LLNA and other current state-of-the-art approaches. Numerous discrete *in vitro* endpoint assays are in development to predict the hazard (sensitizer/non-sensitizer) and potency of chemicals, but there is a lack of integration to recapitulate the sequence of toxicological events leading to sensitization. In this presentation, the new integrated approach using human skin equivalent models that our interdisciplinary, cross-institutional Singaporean team have adopted to investigate chemical-induced ACD will be described. This approach is mechanistically grounded and quantitatively driven, and is in line with the vision of next generation safety assessments. To date human skin equivalent models combined with *in vitro* partition and diffusion coefficients for dinitrochlorobenzene (DNCB) have been used for PBTK modeling. Comparison of simulated and experimental data from the literature shows reasonable recapitulation of its local and systemic exposure profiles. In addition, a number skin proteins that underwent covalent adduction by DNCB were identified using a novel cellular thermal shift assay (CETSA) and are currently being evaluated for their immunogenicity.

III-15-701

Advances in *in vitro* and *in silico* tools for toxicokinetic dose modeling and predictive toxicology

Barbara Wetmore

ORD, U.S. EPA, Research Triangle Park, NC, United States
wetmore.barbara@epa.gov

Recent advances *in vitro* assays, *in silico* tools, and systems biology approaches provide opportunities for refined mechanistic understanding for chemical safety assessment that will ultimately lead to reduced reliance on animal-based methods. With the U.S. commercial chemical landscape encompassing thousands of chemicals with limited data, safety assessment strategies that reliably predict *in vivo* systemic exposures and subsequent *in vivo* effects efficiently are a priority. Quantitative *in vitro-in vivo* extrapolation (QIVIVE) is a methodology that facilitates the explicit and quantitative application of *in vitro* experimental data and *in silico* modeling to predict *in vivo* system behaviors and can be applied to predict chemical toxicokinetics, toxicodynamics and also population variability. Tiered strategies that incorporate sufficient information to reliably inform the relevant decision context will facilitate acceptance of these alternative data streams for safety assessments.

This abstract does not necessarily reflect U.S. EPA policy.

Session III-16: Innovations in Drug Development

Co-Chairs
Lewis Kinter, GLP Scientific Consulting, LLC, Unionville, PA, United States
Kate Harris, NC3Rs, London, United Kingdom

III-16-385

Science, technology, & 3Rs in pharmaceutical development

Lewis Kinter[1,2], Joseph DeGeorge[3], Lida Anestidou[4], Cory Brayton[5], Lawrence Schook[6] and Margaret Landi[7]

[1]GLP Scientific Consulting, LLC, Unionville, PA, United States; [2]Pharmacology & Toxicology, Michigan State University, College of Osteopathic Medicine, East Lansing, MI, United States; [3]Safety Assessment and Laboratory Animal Resources, Merck Research Laboratories, West Point, PA, United States; [4]Institute for Laboratory Animal Research, Washington, DC, United States; [5]Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD, United States; [6]Animal Sciences and Radiology, University of Illinois at Urbana-Champaign, Urbana, IL, United States; [7]GlaxoSmithKline, King of Prussia, PA, United States
lewis@glpsci.com

Drug development is arguably the oldest of the applied life sciences, dating back at least 3500 years; animals have only been used human surrogates in pharmaceutical testing for roughly the past 100 years. We identify 5 transformations of drug development and animal testing dating from 19th century: Anesthesia/antisepsis, medicinal chemistry, recombinant DNA, regulatory toxicology, and targeted drug discovery. Each transformation resulted from a unique cluster of new science and enabling technologies that coalesced over a period of 20-40 years (Kinter and De George, 2016). We leverage the knowledge gained from those historic transformations to identify missing elements from emerging clusters, e.g., computational biology, 3D multicellular constructs, and CRISPR, that could become the next big transformations in drug development and leading to further 3Rs advances.

Reference

Kinter, L. B. and De George, J. J. (2016). Scientific knowledge and technology, animal experimentation, and pharmaceutical development. *ILAR J 57*, 101-108.

III-16-647

The development of *in silico* toxicology protocols

Glenn Myatt, Dave Bower, Kevin Cross, Catrin Hasselgren, Scott Miller and Donald Quigley

Leadscope, Inc., Columbus, OH, United States
gmyatt@leadscope.com

This project is generating a series of protocols to support the prediction of a series of major toxicological endpoints (e.g., genetic toxicity, carcinogenicity, acute toxicity, reproductive and development toxicity). These protocols are being developed through an international cross-industry consortium to reflect the state-of-the art in computational toxicology for hazard identification and characterization. The consortium is led by Leadscope and includes 45 organizations from international regulatory agencies and government research laboratories in the US, Canada, Japan, and Europe as well as large companies from the various industrial sectors (e.g., pharmaceutical, food, cosmetics, agrochemicals), academic groups and other stakeholders. The protocols will ensure any *in silico* toxicological assessments are performed in a consistent, repeatable, well-documented and defendable manner. This includes how to assess the reliability and relevance of data/predictions, how an expert review of the results may be performed and how an overall assessment may be performed based on the weight-of-the-evidence.

III-16-315

Advanced engineered tissues for replicating first pass metabolism on chip

Vincenza De Gregorio[1], Brunella Corrado[2], Giorgia Imparato[3], Francesco Urciuolo[2] and Paolo Netti[2]

[1]Center for Advanced Biomaterials for Healthcare at CRIB, Istituto Italiano di Tecnologia, Napoli, Italy, [2]Center for Advanced Biomaterials for Healthcare at CRIB, Istituto Italiano di Tecnologia, Naples, Italy; [3]Istituto Italiano di Tecnologia, Naples, Italy
vincenza.degregorio@iit.it

In this work, we developed an innovative gut-liver-on-chip system useful to predict oral drug administration and first pass metabolism. The two main organs involved in the first pass metabolism are the liver and the intestine. First-pass effects consist mainly in the reduction of bioavailability of drugs and xenobiotics. The prediction of this mechanism is important both for the development of new substances, but also for toxicity testing. For this purpose, we designed a microfluidic device which interconnect 3D human intestinal equivalent (3D-HIE) and HepG2-microtissues, recapitulating the intestinal and hepatic first-pass effect mechanism of ethanol. 3D-HIE were obtained by bottom up approach, using intestinal microtissues moulded into a maturation chamber and HepG2-μTPs were obtained by dynamic cell seeding of Hepg2 and gelatin porous microsphere in a spinner flask bioreactor.

Reference

Imparato, G., Urciuolo, F. Casale, C. and Netti, P. A. (2013). The role of microscaffold properties in controlling the collagen assembly in 3D dermis equivalent using modular tissue engineering. *Biomaterials 34*, 7851-7861.

III-16-591

CRACK IT: Applying the 3Rs through open innovation

Kate Harris, Cathy Vickers and Anthony Holmes

National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, United Kingdom
kate.harris@nc3rs.org.uk

The bioscience industries are increasingly seeking solutions from the academic and SME sectors for major challenges to their business. Often these challenges relate to the use of animals, for example the problem of predicting clinical efficacy and safety from preclinical studies. The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has responded to this with the launch of CRACK IT, an open innovation platform which connects academia, industry and the SME sector in addressing scientific "Challenges" which if solved will deliver 3Rs (replacement, reduction and refinement of animals in research) and commercial benefits, by improving business processes or developing commercialisable products. Through CRACK IT, we have awarded ~£18M in research contracts to academics and SMEs to develop new models, tools and approaches for the bioscience sector. Here we will introduce the NC3Rs and showcase some of the outputs of the CRACK IT programme.

Session III-17: New Advances in Intestinal Models

Co-Chairs
Julia Yue Cui, University of Washington, Seattle, WA, United States
David L. Eaton, University of Washington, Seattle, WA, United States

III-17-348

Two-dimensional intestinal stem cell-derived organoids as a model for intestinal health

Stefan Vaessen[1], Jean Paul Ten Klooster[1], Saertje Verkoeijen[1], Nikkie Van der Wielen[2], Jocelijn Meijerink[2], Renger Witkamp[2], Cyrille Krul[1] and Raymond Pieters[1]

[1]University of Applied Sciences Utrecht, Utrecht, The Netherlands; [2]Wageningen University & Research Centre, Wageningen, The Netherlands

stefan.vaessen@hu.nl

We developed a two-dimensional (2D) intestinal stem cell-derived organoid model with both crypt and differentiated villus cells (enterocytes, goblet cells and enteroendocrine cells) to study safety and efficacy of compounds. Organoids were grown from mouse duodenal, jejunal and ileal tissue. We show that these 2D organoid cultures maintain location-specific gene expression and responses, e.g. that the artificial sweetener rebaudioside A (derived from Stevia) stimulates production of GLP1 specifically by ileal enteroendocrine cells. Furthermore, we are able to grow 2D intestinal organoid cultures in a transwell format to confluent monolayers with build-up of electrical resistance, low FD4 leakage and responsiveness to cytokines. Results indicate that our 2D intestinal organoid model allows us to study the effects of compounds beyond existing possibilities with standard epithelial cell lines. We are currently exploring the potential of using this culture method to investigate the intestinal uptake of pharmaceuticals.

Reference

van der Wielen, N., Ten Klooster, J. P., Muckenschnabl, S. et al. (2016). The noncaloric sweetener rebaudioside a stimulates glucagon-like peptide 1 release and increases enteroendocrine cell numbers in 2-dimensional mouse organoids derived from different locations of the intestine. *J Nutr 146*, 2429-2435.

III-17-279

Gut on-a-chip: Towards a more physiological and predictive human intestinal barrier model

Evita van de Steeg, Pascalle van Loo, Lianne Stevens, Simon Folkertsma, Steven Erpelinck, Angelique Speulman, Mariska Grollers, Irene Nooijen and Heleen Wortelboer

TNO, Zeist, The Netherlands

evita.vandesteeg@tno.nl

The aim of this study was to generate a physiological and high translational human *in vitro* intestinal barrier model. We successfully applied human intestinal tissue into the InTESTine™ two-compartmental disposable device suitable for standard 6- or 24-well plate format. This set-up is suitable to study (regional differences in) processes that determine drug absorption, including intestinal wall metabolism, food-drug effects, mucus interactions and enteroendocrine responses. By applying apical and basolateral fluidics, an increase in the viability and functionality of the intestinal tissue, up to 24 h of incubation, was demonstrated by a low LDH leakage combined with proper transport and metabolic functionality of the tissue. In conclusion, the InTESTine platform can be applied as a reliable tool for the assessment of processes that determine human intestinal permeability as well as for host-microbe-immune responses when mounted in the microfluidic device.

III-17-102

Cyst-structure formation of biliary epithelial cells (BECs) using PDMS-based microstructure for potential biliary network *in vitro*

Astia Safitri[1], Marie Shinohara[2], Yasushi Miura[3], Mathieu Danoy[4], Minoru Tanaka[5], Miyajima Atsushi[6] and Yasuyuki Sakai[7]

[1]Bioengineering, University of Tokyo, Tokyo, Japan; [2]Chemical System Engineering, Institute of Industrial Science, University of Tokyo, Tokyo, Japan; [3]Waseda University, Tokyo, Japan; [4]LIMMS, IIS, University of Tokyo, Tokyo, Japan; [5]University of Tokyo, Tokyo, Japan; [6]Institute of Molecular and Cellular Bioscience, University of Tokyo, Tokyo, Japan; [7]Istitute of Industrial Science, University of Tokyo, Tokyo, Japan

astiars@iis.u-tokyo.ac.jp

The biliary network establishment *in vitro* is essential in liver cell-based assays for chemicals and drugs metabolism (Katsuda et al., 2013; Seldatow et al., 2013; Han et al., 2013). The cyst-structure assembled by biliary epithelial cells (BECs) (Ogawa et al., 2015; Tanimiz et al., 2007) reported as main building block of biliary network from ductal plate during fetal morphogenesis (Vestentoft et al., 2011; Takashima et al., 2015). It has been commonly cultured in matrigel-embed culture which inappropriately maintain the productivity, homogeneity, and post-treatment of cyst-structure (Ogawa et al., 2015; Tanimiz et al., 2007). This study proposes a new method for cyst-structure establishment using the honeycomb PDMS-based microstructure (Shinohara et al., 2013; Goral et al., 2015). The primary mouse BECs were seeded on the various sizes of honeycomb microwells as well as matrigel supplementations. Results proved that the dimension importantly role in cyst formation (46 μm-size) and supplementation overcome matrigel over-usage. The cytological morphologies, bile acid transportation, and gene expression of the cysts are confirmed the basic function of biliary network. Our method is expected to contribute liver tissue formation for cell-based assay.

References

Goral, V. N., Au, S. H., Faris, R. A. and Yuen, P. K. (2015). Methods for advanced hepatocyte cell culture in microwells utilizing air bubbles. *Lab Chip 15*, 1032-1037.

Han, Y., Glaser, S., Meng, F. et al. (2013). Recent advances in the morphological and functional heterogeneity of the biliary epithelium. *Exp Biol Med (Maywood) 238*, 549-565.

Katsuda, T., Kojima, N., Ochiya, T. and Sakai, Y. (2013). Biliary epithelial cells play an essential role in the reconstruction of hepatic tissue with a functional bile ductular network. *Tissue Eng Part A 19*, 2402-2411.

Ogawa, M., Ogawa, S., Bear, C. E. et al. (2015). Directed differentiation of cholangiocytes from human pluripotent stem cells. *Nat Biotechnol 33*, 853-861.

Soldatow, V. Y., Lecluyse, E. L., Griffith, L. G. and Rusyn, I. (2013). In vitro models for liver toxicity testing. *Toxicol Res (Camb) 2*, 23-39.

Takashima, Y., Terada, M., Kawabata, M. and Suzuki, A. (2015). Dynamic three-dimensional morphogenesis of intrahepatic bile ducts in mouse liver development. *Hepatology 61*, 1003-1011.

Tanimizu, N., Miyajima, A. and Mostov, K. E. (2007). Liver progenitor cells develop cholangiocyte-type epithelial polarity in three-dimensional culture. *Mol Biol Cell 18*, 1472-1479.

Vestentoft, P. S., Jelnes, P., Hopkinson, B. M. et al. (2011). Three-dimensional reconstructions of intrahepatic bile duct tubulogenesis in human liver. *BMC Dev Biol 11*, 56.

III-17-601

Use of 3D human small intestinal tissue model (EpiIntestinal™) as an alternate to animal testing to predict drug-induced gastrointestinal (GI) toxicity

Seyoum Ayehunie[1], Timothy Landry[1], Zachary Stevens[1], Matthew Wagoner[2], Patrick Hayden[1] and Mitchell Klausner[1]

[1]MatTek Corporation, Ashland, MA, United States; [2]AstraZeneca, Watham, MA, United States

sayehunie@mattek.com

The utility of an *in vitro* primary human cell based small intestinal 3D tissue (SMI) model as an investigational tool for drug induced GI toxicity was evaluated. A blinded study was performed using 8 therapeutic compounds (5 problematic and 3 well tolerated drugs in humans) for which dog and rat toxicity studies were not predictive of human outcome. MTT viability, barrier integrity using transepithelial electrical resistance (TEER) measurements and histology were used as end points. Results showed that the SMI tissue detected drug induced reduction in TEER in 5/5 of the problematic drugs at concentrations within or below 30x clinical C_{max}. Importantly, the SMI system showed no effect within 1,000x clinical exposure levels for the three well-tolerated drugs. Overall, the use of TEER and histology as endpoints make the SMI tissue a sensitive predictive tool to assess drug-induced GI toxicity. The use of the SMI tissues for GI toxicity testing is cost effective and reduces animal use.

III-17-96

Human gut-on-a-chip as a model for bioavailability and biotransformation studies

Hans Bouwmeester[1,2], Kornphimol Kulthong[1,2], Loes Duivenvoorde[1] and Meike Van der Zande[1]

[1]RIKILT – Wageningen Research, The Netherlands; [2]Laboratory of Toxicology Wageningen University, Wageningen, The Netherlands

hans.bouwmeester@wur.nl

We developed a gut-on-a-chip model for kinetic absorption and biotransformation studies to replace or refine animal models. The chip constitutes two chambers separated by a porous membrane on which Caco-2 intestinal cells were grown. We characterised the model versus a static Transwell® model. Confocal microscopy and cell layer integrity evaluation indicated a decrease in the proliferation and differentiation time from 21 days in the Transwell to 7 days in the chip. Differentiation was more pronounced in the chip. Translocation efficacy was measured using hrGC-MS after exposure to 17 dioxin congeners for 24 h. Similar translocation profiles between the chip and Transwell were observed, but translocation percentage was ~5x lower in the chip. qPCRs of CYP1A1 and CYP1A2 expression in both systems, revealed ~390 fold change induction of CYP1A1, but no induction of CYP1A2. Subsequently, we combined Caco-2 and HT29-MTX cells on the apical side and hMVEC-d endothelial cells on the basolateral side of the membrane. Confocal microscopy analysis shows successful integration of the triple cell culture, we are currently working on further evaluation of this model.

III-17-444

Replacement of goat xenobiotic metabolism studies by use of rumen simulation technique (RUSITEC)

Barbara Birk[1], Alexander Staehle[2], Mathias Meier[2], Dorothee Funk[2], Gerhard Breves[3] and Harald Seulberger[2]

[1]BASF SE, Ludwigshafen, Germany; [2]BASF SE, Limburgerhof, Germany; [3]TiHo Hannover, Hanover, Germany

barbara.birk@basf.com

For registration of plant protection products 14C-metabolism studies are required. Often questions occur: 1) Are the observed metabolites ruminant specific? 2) Are ruminants able to cleave plant specific metabolites? In the past additional *in vivo* goat metabolism studies beyond OECD 503 needed to be performed. Rumen simulation technique (RUSITEC) was used as a potential method to simulate the rumen *in vitro*. Fresh rumen fluid was incubated *in vitro* 7 days by RUSITEC. The vitality and metabolic behavior was tested. The pH and the redox potential kept constant in the physiological range over time. Radio-HPLC showed that Triazole-alanine was cleaved to 1,2,4-triazole, while Triazole-acetic acid and Triazole-lactic acid were stable. This is in accordance with *in vivo* studies performed ruminants according to OECD 503 and 505. Moreover, a complete degradation of glycosides (e.g. 12 C-Polydatin) to the respective aglycon was also shown. BASF will replace *in vivo* animal studies on ruminant metabolism studies beyond OECD 503 by performing RUSITEC studies.

Theme III: Innovative Models for Safety and Efficacy

Poster Presentations

Innovative Models for Safety and Efficacy – Occular Models

III-113

Expansion of the applicability domain in the Short Time Exposure (STE) test method for assessing eye irritation potential

Takayuki Abo[1], Takuo Yuki[1], Daisuke Araki[1], Yutaka Takahashi[1], Javier Avalos[2], Hitoshi Sakaguchi[1] and Hiroshi Itagaki[3]

[1]Kao Corporation, Tochigi, Japan; [2]Kao USA Inc., Cincinnati, OH, United States; [3]Yokohama National University, Yokohama, Japan
abo.takayuki@kao.co.jp

The Short Time Exposure (STE) test method has been adopted as test guideline 491 by OECD. When used to identify chemicals as GHS "No Category" for eye irritation, one limitation is highly volatile substances (vapor pressure > 6kPa, 25°C). Substances like acetone have produced false negative results when saline was used as the solvent in the STE test method. However, these substances were correctly predicted as eye irritants when mineral oil was used as the solvent. Solids except for surfactants were considered another limitation for the STE test method and were not originally evaluated. Solids like imidazole, which was classified as GHS irritant category, were correctly predicted as irritants when evaluated in the STE test method. As a result, the number of correctly evaluated substances was increased from 102 to 124 by incorporating mineral oil as a solvent and reconsidering solids in STE test method Summary Review Document, which would increase the applicability domain.

III-192

BCOP-LLBO method – results of CON4EI, ALT4EI, and validation study

Sandra Verstraelen[1], Els Adriaens[2] and An R. Van Rompay[1]

[1]Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium; [2]Adriaens Consulting bvba, Aalter, Belgium
sandra.verstraelen@vito.be

The CEFIC-LRI-AIMT6-VITO CON4EI project (CONsortium for *in vitro* Eye Irritation testing strategy; 2015-2016) assessed the reliability of 7 *in vitro* test methods and computational models as well as established tiered-testing strategies. BCOP-LLBO is incorporated in one of the testing strategies together with SkinEthic™ HCE EIT or EpiOcular™ EIT to address the whole spectra of eye irritation potential. Currently, a validation study for the BCOP-LLBO method (sponsored by CEFIC, 2016) is finalized to demonstrate the utility and the applicability of this device to be integrated in OECD Test Guideline 437. Furthermore, in the LNE-sponsored ALT4EI project (ALTernatives for Eye Irritation; 2017) remaining data gaps for the BCOP-LLBO method will be filled to strengthen the data set. The BCOP-LLBO results of these projects will be presented.

III-264

Allergic sensitization leads to inadequate anti-viral immune response in small airways *ex vivo*

Olga Danov, Lisa Laßwitz, Helena Obernolte, Christina Hesse, Armin Braun, Sabine Wronski and Katherina Sewald

Fraunhofer ITEM, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Hannover, Germany

olga.danov@item.fraunhofer.de

Asthmatic patients suffer from virus-induced exacerbations mainly caused by human rhinovirus (HRV). Therefore, in this study the impact of an asthmatic background on the immune response to HRV was investigated in lung tissue (PCLS) of HDM-sensitized mice after *ex vivo* infection. *In vivo* HDM sensitization resulted in a T_H-2/T_H-17 dominated microenvironment in lung tissue which maintained *ex vivo*. HRV1B infection of sham-sensitized PCLS lead to a significantly higher secretion of IFN-γ (480%), IP-10 (1625%), IL-17A (1300%) and TNF-α (200%). Contrary to this, HRV1B induced only minor and impaired immune response in HDM-sensitized PCLS with a significantly attenuated secretion of IFN-γ (206%), IP-10(133%) and IL-17A (140%). Interestingly, IL-4 was further exacerbated by HRV1B in PCLS of HDM-sensitized mice. Further studies using this model can reveal pathways involved in the mechanisms of virus-induced exacerbation of asthma.

III-275

Inflammatory cytokine production evaluation in 3D human corneal-like epithelial tissue model for eye irritation prediction

Marize Campos Valadares, Artur Christian G. da Silva, Elisandra Gava and Adrienny Rodrigues Chialchia

Laboratorio de Farmacologia e Toxicologia Celular, Faculdade de Farmacia, Universidade Federal de Goias, Goiânia, Brazil

marizeufg@gmail.com

Eye irritation is a mandatory parameter in human risk and safety evaluations of chemicals. In this sense, a non-animal alternative method for reliable and accurate assessment of ocular toxicity in man are needed. In this work, we used a 3D human corneal-like epithelial tissue model to investigate inflammatory cytokine profile after exposure to chemicals. As a first step, the cytotoxicity of the chemicals (3 non-irritants and 7 eye irritants distributed in GHS Category 1, 2A and 2B), after 24 h of exposure, was evaluated to obtain 80% of cell viability (CV80). Then, the 3D human corneal tissue was exposed to chemicals at CV80 for 24 h and supernatants and cell lysates were obtained for measuring the inflammatory cytokines (IL-6, IL-8, IL-10, IL-1β, TNF and IL-12p70). Among of these cytokines, the IL-1 was markedly changed after exposure. The higher levels of intracellular IL-1β were found after exposure to chemicals of GHS Category 1, followed by Category 2. Thus, these findings showed that IL-1β production in the 3D tissue can contribute to classifying the ocular irritation potential of chemicals without the use of animals to meet regulatory testing requirements.

References

Adriaens, E., Barroso, J., Eskes, C. et al. (2014). Retrospective analysis of the Draize test for serious eye damage/eye irritation: Importance of understanding the in vivo endpoints under UN GHS/EU CLP for the development and evaluation of in vitro test methods. *Arch Toxicol 88*, 701-723.

Barroso, J., Pfannenbecker, U., Adriaens, E. et al. (2017). Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: The Draize eye test Reference Database (DRD). *Arch Toxicol 91*, 521-547.

III-366

Development of the *in vitro* assay for evaluating weak eye irritation

Hajime Kojima[1], Noriko Hiramatsu[2], Yoshinao Katou[3] and Naoki Yamamoto[2]

[1]National Institute of Health Sciences, Tokyo, Japan; [2]Laboratory of Molecularbiology and Histochemistry, Fujita Health University Institute of Joint Research, Toyoake, Japan; [3]General Institute, Nippon Menard Cosmetic Co., Ltd., Nagoya, Japan

h-kojima@nihs.go.jp

Differences in reversibility of cornea damages could not be distinguished in previously developed non-animal alternative eye irritation methods. Whether the eye damage caused in reversible or irreversible is an important hazard assessment criterion such as GHS category 2A or 2B. Based on the performance standard in OECD TG 492, the protocol was developed using a three-dimensional cornea model with cloned iHCE-NY1 cell line, which was derived from corneal epithelial cells isolated from human corneal tissue and transfected with immortalized gene. To evaluate reversible cytotoxicity of test substances, the MTT assay and pathological finding were examined with the cornea model on day 1, 7 and 14 of post-culture. These results address the post-incubation of the cornea model after applying test chemicals may be useful to evaluate reversible cornea damage.

III-534

OECD TG 492 performance standard based multi-laboratory validation study for a new *in vitro* eye irritation test using the reconstructed human corneal epithelial model, MCTT HCE

Soojin Ha[1], Mi-Sook Jung[2], Kyung-Wook Yeo[3], Jin-Sik Kim[4], Su-Hyon Lee[5] and Kyung-Min Lim[1]

[1]College of Pharmacy, Ewha Womans University, Seoul, South Korea; [2]Pharmacology Efficacy Team, Biotoxtech Co., Ltd, O-Chang, South Korea; [3]Healthcare R&D Center, Korea Testing and Research Institute, Hwa-Soon, South Korea; [4]Korea Conformity Laboratories, Song-do, Incheon, South Korea; [5]Biosolution Co., Ltd., Seoul, South Korea

hakatherine11@gmail.com

MCTT HCE was developed in Korea to replace the rabbit Draize eye irritation test (EIT). Its pre-validation study revealed a limitation on the sensitivity for solid substances. To improve this, we amended the protocol for solid substance wherein the treatment time was increased from 10 minutes to 3 hours. With this revision, the proficiency test for 15 reference chemicals was carried out in 4 GLP laboratories prior to the main test. The results showed that 13 out of 15 substances matched the determination of ocular irritancy (86.67% between-laboratory agreement). Following the successful proficiency test, the validation study was conducted for 30 full reference chemicals according to the OECD TG 492 performance standard to evaluate the predictive capacity of the MCTT HCE EIT, also to establish its reliability and reproducibility. Here, we present the results of the MCTT HCE EIT validation study.

This research was supported by grant (16182MFDS522) from the Ministry of Food & Drug Safety of Korea.

III-566

Literature review on *in vitro* and alternative developmental neurotoxicity (DNT) testing methods

Stefan Masjosthusmann[1], Ellen Fritsche[1], Jenny Baumann[1], Lieve Geerts[2] and Hilda Witters[2]

[1]Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany; [2]UNIT Environmental Risk & Health, Flemish Institute for Technological Research, Mol, Belgium

Stefan.Masjosthusmann@iuf-duesseldorf.de

A promising approach for future DNT testing that will allow the identification of potential DNT compounds and reduce *in vivo* testing is the use of alternative methods integrated in a DNT test battery. The goal of this review was to comprehensively search and evaluate alternative methods to be used in a test battery. We retrieved and evaluated a variety of methods (from stem cells to zebrafish larvae) suitable to identify adverse effects of chemicals during different stages of neurodevelopment. In general, existing method need a defined biological application domain and international standardization. To advance DNT testing, a battery needs to be set up to perform compound screening across a set of promising assays that represent major stages and processes of neurodevelopment. Such data can be used to validate the test battery and support international regulatory assessments or compound prioritization in favor of a reduction of rodent DNT testing.

Reference

Fritsche, E., Alm, H., Baumann, J. et al. (2015). Literature review on in vitro and alternative developmental neurotoxicity (DNT) testing methods. *EFSA Supporting Publication 2015: EN-778*.

III-626

Proposal for an *in vitro* strategy for ocular irritation assessment: Emergence of a new category for personal care formulated products

Sophie Catoire, Andrea Cano and Hervé Ficheux

Thor Personal Care, Compiegne, France

sophie.catoire@thorpc.fr

Assessment of eye tolerance of personal care ingredients and formulated products is strongly required by regulatory authorities who encourage the companies to implement relevant toxicological studies in this field. Currently, the five accepted methods by OECD were, at first, designed for raw material toxicity evaluation. Nevertheless, the validated methods must be adapted in order to meet the specific needs for personal care formulations (leave-on and rinse-off products). Our laboratory has implemented an *in vitro* method to assess formulated personal care products based on a calculation of simplified *in vitro* score allowing to identify four categories (non irritant, slightly, moderate and irritant). Our *in vitro* eye irritation testing strategy based on combination of tests is presented in this poster. This new approach is illustrated through different practical examples and revisits the non irritant category.

III-654

Molecular mechanisms of corneal oxidative stress: *In vitro* reconstructed human corneal tissue model (EpiCorneal™)

Yuila Kaluzhny, Miriam Kinuthia, Thoa Truong, Alena Plotkin, Patrick Hayden and Mitchell Klausner

MatTek Corporation, Ashland, MA, United States

ykaluzhny@mattek.com

The cornea is exposed to UV and oxidative damage that plays a role in diseases including dry eye disease (DED). Physiologically relevant human *in vitro* models are needed for ocular research. We studied oxidative stress (OS) and DED using an *in vitro* model comprised of normal human corneal epithelial cells cultured at the air-liquid interface. OS was generated by UV, H2O2 or desiccating stress conditions (DSC) to simulate DED. Reactive oxygen species (ROS), cytokine release, barrier function, tissue viability, histology, and gene expression were evaluated. UV and DSC caused increased ROS, release of IL-8 and upregulation of proinflammatory cytokine and enzyme genes. Application of lubricant gel drops improved tissue morphology and barrier function, but didn't affect cytokine release or gene expression. The EpiCorneal model avoids species extrapolation, is cost effective and reproducible, and will facilitate screening and optimization of pharmaceutical formulations.

III-704

Test the delivery temperature for stability of 3D reconstructed human corneal epithelium tissue model, SoluEye™

Su Hyon Lee[1], Seol Yeong Kim[1], Hye Soo Kim[1] and Kyoung Min Lim[2]

[1]Biosolution Inc., Seoul, South Korea; [2]Ewha Womans University, Seoul, South Korea

rms@biosolutions.co.kr

Many efforts are being made to develop new alternative *in vitro* test methods for the eye irritation test. The SoluEye™ is a new 3D reconstructed human cornea epithelium model for alternative to animal testing for the ocular irritancy.

The need for non-animal ocular test method in neighboring Asian countries, including Japan and China, is increasing. Therefore, we investigated the delivery temperature for the stability of SouEye™ model. We conducted endpoint analysis including transepithelial electrical resistance (TEER), effective time-50 (ET-50) for Triton X-100, Histology and eye irritation test using the SoluEye™ . By increasing the time for shipment from 12 hours to 72 hours, SoluEye was confirmed to be stable at 4 degrees and 18 degrees.

This research was supported by grants (13172MFDS987 and 16182MFDS522) from the Ministry of Food & Drug Safety of Korea.

References

Jung, K. M., Lee, S. H., Ryu, Y. H. et al. (2011). A new 3D reconstituted human corneal epithelium model as an alternative method for the eye irritation test. *Toxicol In Vitro 25*, 403-410.

Kaluzhny, Y., Kandárová, H., Handa, Y. et al. (2015). The EpiOcular Eye Irritation Test (EIT) for hazard identification and labelling of eye irritating chemicals: Protocol optimization for solid materials and the results after extended shipment. *Altern Lab Anim 43*, 101-127.

Innovative Models for Safety and Efficacy – Respiratory Models

III-183

In vitro model for the prediction of respiratory sensitization of inhaled chemicals

Tommaso Serchi[1], Aline Chary[1], Sebastien Cambier[1], Elisa Moschini[1], Servane Contal[1], Jennifer Hennen[2], Brunhilde Blömeke[2] and Arno Gutleb[1]

[1]Environmental Research and Innovation, List, Belvaux, Luxembourg; [2]Department of Environmental Toxicology, Trier University, Trier, Germany

tommaso.serchi@list.lu

Occupational exposure to chemicals such as acid anhydrides can lead to the development of respiratory allergies. To mimic the alveolar-capillary barrier we developed a 3D *in vitro* model grown at the air liquid interface including alveolar type II epithelial cells (A549), endothelial cells (EA.hy926), macrophages like cells (PMA differentiated THP-1) and dendritic like cells (DC) (non-differentiated THP-1).

The model was exposed at the ALI to trimellitic anhydride (TMA) and phthalic anhydride (PA), which are chemical respiratory sensitizers, and to acrolein (Acr), which is a respiratory irritant.

Viability of the co-culture was assessed 24 h after exposure and concentration leading to 75% of viability of the tissue were selected for the following experiments. Exposure to PA and TMA lead to the upregulation of CD54 and TSLPR on DC cells as compared to Acr or vehicle controls, demonstrating that this model can discriminate *in vitro* between respiratory sensitizers and irritants.

III-201

Integrating alternative approaches to replace animals in inhalation toxicity testing

Dave Allen[1], Daniel Wilson[2], Jon Hotchkiss[2], Steven Morefield[1], Warren Casey[3] and Amy Clippinger[4]

[1]ILS, Morrisville, NC, United States; [2]Dow Chemical Company, Midland, MI, United States; [3]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, United States; [4]PETA International Science Consortium Ltd., London, United Kingdom

dallen@ils-inc.com

Non-animal approaches for acute inhalation toxicity testing must address the multiple mechanisms associated with acute local and systemic toxicity of inhaled substances. A September 2016 workshop co-hosted by NICEATM and PISC resulted in recommendations and prioritizations of actions needed to achieve regulatory acceptance of *in vitro* and *in silico* approaches. This presentation will summarize the workshop and report on the status of subsequent activities. These activities will more fully characterize the state of the science of non-animal alternatives, catalog the available inhalation toxicology data resources for developing and evaluating alternative approaches, and establish the steps necessary to implement an integrated testing framework to reduce and replace animal use for hazard-based acute inhalation toxicity testing.

This was funded with U.S. Federal funds from NIEHS/NIH/HHS under Contract HHSN27320140003C.

III-215

Evaluation of EGFR induced on- and off-target effects in a microfluidic 3D human lung tumour – Full thickness skin co-culture model

Juliane Hübner[1], Marian Raschke[2], Isabel Rütschle[1], Susanne Schnurre[2], Sarah Gräßle[1], Ilka Maschmeyer[1], Uwe Marx[1] and Thomas Steger-Hartmann[2]

[1]TissUse GmbH, Berlin, Germany; [2]Investigational Toxicology, Bayer AG, Berlin, Germany
juliane.huebner@tissuse.com

Microphysiological systems are increasingly contributing to the preclinical prediction of mode of action and adverse outcome pathways of new chemical and biological entities. The recent advent of robust human multi-organ-chip systems enables the establishment of co-cultures of human drug target tissues with healthy organ equivalents prone to off-target effects of the respective drug.

Here we established a chip-based 6-day co-culture composed of human mucoepidermoid carcinoma H292 cell-based lung tumour spheroids and human skin equivalents. We investigated the impact of repeated Cetuximab exposure on the systemic behaviour of the co-culture and on individual tissue responses. We compared on-target antibody effects with response data for Afatinib – a small molecule benchmark drug. Finally, we investigated off-target effects on healthy skin equivalent.

This co-culture has the potential to provide a platform for evaluation of the therapeutic window of drug candidates.

III-228

A promising tool for acute inhalation toxicology *in vitro*

Emilie Da Silva[1,2], Karin Sørig Hougaard[1] and Jorid Birkelund Sørli[1]

[1]Toxicology, National Research Center for the Working Environment, Copenhagen, Denmark; [2]Environment, Technical University of Denmark, Kgs. Lyngby, Denmark
eds@nrcwe.dk

The constrained drop surfactometer (CDS) is a promising tool for acute inhalation toxicology *in vitro*. Chemical substances entering the market as well as particles emitted in the environment are human health hazards. Testing their toxicity upon inhalation is crucial. However, the only method currently validated is animal exposure to an aerosol of particles for up to 4 hours, involving potential severe suffering. Therefore, we developed an innovative *in vitro* system capable of assessing lung toxicity of dry powders and liquid aerosols under physiologically relevant conditions. The CDS method is less time consuming, less expensive and more ethically sound than the currently recommended OECD test guideline. This system will contribute to reducing the use of animals.

III-270

In vitro evaluation of novel antibiotics against *Pseudomonas aeruginosa* infection on human airway epithelia

Carole Bertinetti, Ophelie Verbeke, Mireille Caulfuty, Paul Alouani, Ludovic Wiszniewski, Song Huang and Samuel Constant

Epithelix, Plan-les-Ouates, Switzerland
carole.bertinetti@epithelix.com

Pseudomonas aeruginosa (PA) infection are increasingly associated with acute exacerbations in chronic obstructive pulmonary disease (COPD). We report herein the use of 3D airway epithelia, MucilAir™ , made of a mixture of primary cells from 14 human donors for screening novel antibiotics upon PA infection.

PA were inoculated ($1E^{+02}$ CFU/0.33 cm^2) on fully differentiated MucilAir™ in presence or absence of mucus, with or without Meronem (50 μg/ml). PA growth, cilia beating frequency (CBF), cytotoxicity (LDH) and tissue integrity (TEER) were assessed daily during 4 days.

A higher proliferation rate of PA in absence of mucus was observed, highlighting the protective role of mucus containing antimicrobial peptides. Meronem efficiently inhibited both growth of PA and the cytotoxicity (LDH) and restored the impaired barrier function (TEER) in a time dependent manner.

These results demonstrate that MucilAir™ is a robust, reliable and relevant tool for drug development against PA infection.

III-272

The use of *in vitro* human airway epithelia for the development of novel antivirals

Bernadett Boda[1], Rosy Bonfante[1], Sacha Benaoudia[1], Song Huang[1], Laurent Kaiser[2], Caroline Tapparel[2] and Samuel Constant[1]

[1]Epithelix, Plan-les-Ouates, Switzerland; [2]Geneva University Hospitals, Laboratory of Virology, Geneva, Switzerland
bernadett.boda@epithelix.com

Respiratory viral infections cause mild to severe diseases worldwide. We report herein the use of 3D epithelia made of human primary nasal cells, MucilAir™ , for viral drug screening.

Clinically relevant Rhinovirus (A16, C15), Enterovirus (EV68) and Influenza A virus (H1N1, H3N2) strains were added to fully differentiated MucilAir™ -Pool (mix of 14 donors). Released viral genome copy number, overall mucin secretion, cilia beating frequency, MCC and tissue integrity were assessed daily during 4 days.

MucilAir™ showed high rate of replication for all tested viruses, including difficult-to-culture HRV-C15. Rupintrivir efficiently inhibited the replication of HRV-A16 and HRV-C15 in a dose and time dependent manner and restored MCC impaired by EV68. Oseltamivir reduced the replication of H1N1 and H3N2 and restored the impaired barrier function (TEER).

These results demonstrate that MucilAir™ is a robust, reliable and relevant tool for antiviral drug development.

III-302

Assessment of mitochondrial function following long-term treatment of human bronchial epithelial cells with total particulate matter from a candidate modified-risk tobacco product versus cigarettes

Marco van der Toorn[1], Dominika Malińska[2], Jędrzej Szymański[2], Jarosław Walczak[2], Monika Pril[2], Karolina Drabik[2], Bernadeta Michalska[2], Aleksandra Wojtala[2], Paulina Patalas-Krawczyk[2], Małgorzata Partyka[2], Stephanie Johne[1], Karsta Luettich[1], Manuel Peitsch[1], Julia Hoeng[1], Mariusz Więckowski[2], Joanna Szczepanowska[2] and Jerzy Duszyński[2]

[1]PMI R&D, Philip Morris Products S.A., Neuchâtel, Switzerland; [2]Polish Academy of Sciences, Nencki Institute of Experimental Biology, Warsaw, Poland
Marco.vandertoorn@pmi.com

Reduction of harmful constituents by heating rather than combusting tobacco could reduce the oxidative stress-induced physiology in airway inflammation. We evaluated mitochondrial function in airway epithelial cells following a 12-week exposure to total particulate matter (TPM) from the aerosol of a candidate modified-risk tobacco product, the Tobacco Heating System2.2 (THS2.2) in comparison to TPM from the 3R4F reference cigarette. Endpoints linked to mitochondrial dysfunction, including mitochondrial biogenesis and oxidative stress, were assessed. 3R4F and THS2.2 TPM treatment resulted in decreased mitochondrial mass and level of complexes II, III and IV. Increased proton leakage, expression of NRF1, NRF2 and SOD1 with decreased levels of ROS were observed in cells treated with 3R4F or a 20-fold higher concentration of THS2.2 TPM. Treatment with TPM from THS2.2 had a lower dose-dependent impact on mitochondrial function in comparison with TPM from a combusted tobacco product.

III-389

Towards the characterization of a three-dimensional *in vitro* alveolar model to test nanocarriers translocation

Ana Costa[1,2,3], Cristiane Carvalho-Wodarz[4], Bruno Sarmento[1,2,5], Claus-Michael Lehr[4] and Vítor Seabra[5]

[1]i3S, Instituto de Investigação e Inovação em Saúde, Porto, Portugal; [2]Nanomedicines & Translational Drug Delivery, INEB, Instituto de Engenharia Biomédica, Porto, Portugal; [3]ICBAS, Instituto Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal; [4]Department of Drug Delivery (DDEL), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, Germany; [5]CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal
ana.margarida.costa@ineb.up.pt

3D *in vitro* models reproducing structural and functional features of tissues are of great value to reduce the use of animals. We aim to establish an *in vitro* model of the alveolar epithelium at air-liquid interface, constituted by NCIH441 epithelial cell line (Salomon et al., 2014), a human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) (Hermanns et al., 2004), and THP-1 macrophages.

Epithelial and endothelial cells were seeded, respectively, at apical and basolateral side of a transwell filter (3.0 μm). Mono or co-culture of NCI-H441 cells (250,000 cell/well) showed a transepithelial electrical resistance (TEER) between 140-300 Ohms.cm2 after 8 days. Unexpectedly, transmission electron microscopy showed that NCI-H441 formed multi-layers in both conditions. By reducing the amount of epithelial cells (100,000/transwell) we obtained monolayer in transwells of 3.0 or 0.4 μm, with a TEER of ~100 or 417 ±69 Ohms.cm^2, respectively. The model established at transwell 3.0 μm allows nanoparticle translocations studies, while the one at 0.4 μm pore is suitable to assess epithelial barrier integrity. Particle uptake as well as biocompatibility assays are feasible with both pore sizes.

References

Hermanns, M. I., Unger, R. E., Kehe, K. et al. (2004). Lung epithelial cell lines in coculture with human pulmonary microvascular endothelial cells: Development of an alveolo-capillary barrier in vitro. *Lab Invest 84*, 736-752.

Salomon, J. J., Muchitsch, V. E., Gausterer, J. C. et al. (2014). The cell line NCl-H441 is a useful in vitro model for transport studies of human distal lung epithelial barrier. *Mol Pharmaceutics 11*, 995-1006.

III-497

Development of an *in vitro* alternative method for acute inhalation toxicity testing

Patrick Hayden, Geroge Jackson, Anna Maione and Mitchell Klausner

MatTek Corporation, Ashland, MA, United States
phayden@mattek.com

Knowledge of acute inhalation toxicity potential is important for establishing safety of chemicals. A newly developed *in vitro* toxicity test was evaluated in comparison to established *in vivo* tests. The *in vitro* test exposes EpiAirway™ tissues to chemicals for 3 hrs, followed by measurement of tissue viability. 64 chemicals covering a broad range of toxicity classes, chemical structures and physical properties were evaluated. The results suggest that rat LD_{50} tests, while good for predicting highly toxic chemicals, produce a high percentage of false negative predictions for moderately/slightly toxic or irritating chemicals. The *in vitro* test using the EpiAirway™ human airway model was equal to current rat LD_{50} tests for predicting highly toxic inhaled chemicals, and better than the animal tests for predicting moderately/slightly toxic respiratory irritants. The new *in vitro* testing approach should provide improved protection of human health compared to currently accepted animal tests.

III-522

Modelling the human airways: From physiology to pathology

Lindsay Marshall[1] and Catherine Willett[2]

[1]Research & Toxicology, Humane Society International, London, United Kingdom; [2]Regulatory Toxicology, Risk Assessment and Alternatives, Humane Society of the United States, Gaithersburg, MD, United States
lmarshall@hsi.org

Respiratory disease is increasing; chronic obstructive pulmonary disease is predicted to be the third biggest global killer by 2020. New drugs are needed for many respiratory conditions but candidate molecules that appear promising in animal models do not translate to the clinic. To overcome limitations of animal models for respiratory disease, we developed multi-cellular models of the human airways for disease research (infectious and inflammatory conditions). Co-culture of fibroblasts and epithelial cells at the air-liquid interface produces a typical tight barrier, measured by transepithelial electrical resistance and FITC-dextran permeability. These models were used to mimic bacterial infections characteristically found in cystic fibrosis and to examine electronic cigarette induced damage.

The development of multi-cellular, three-dimensional models of the human airways such as these are of great value to respiratory, and many other fields of health research.

III-646

In silico characterization of peptides from allergenic proteins for the prediction of allergenic potential of proteins

Brunhilde Blömeke[1], Jennifer Hennen[1], Nora Lisa Krutz[1], Carlos Rodriguez[2], Carsten Goebel[3,4], Frank Gerberick[5] and Sebastian Maurer-Stroh[6]

[1]Department of Environmental Toxicology, Trier University, Trier, Germany; [2]The Procter & Gamble Company, Strombeek-Bever, Belgium; [3]The Procter & Gamble Company, Schwalbach am Taunus, Germany; [4]Present address: Coty Professional Beauty, Darmstadt, Germany; [5]The Procter & Gamble Company, Mason, OH, United States; [6]Bioinformatics Institute, Agency for Science, Technology and Research, Singapore
bloemeke@uni-trier.de

Respiratory allergy may occur due to plant extracts with potentially allergenic proteins in consumer products. To induce an allergen-specific IgE response, allergen-derived peptides need to bind to HLA class II molecules and induce $CD4^+$ T cell responses. We studied predicted HLA class II binding characteristics of peptides from 23 proteins and found no differences between allergens and non-allergens. Then we analyzed 426 peptides from different allergenic sources with published data on T cell proliferation in allergic (IgE^+) and non-allergic (IgE^-) subjects. Peptides inducing T cell responses in IgE^+ and IgE^- subjects include more strong HLA class II binders, show sequence similarities with known linear B cell epitopes, and tend to be more conserved and less flexible. Peptides evoking T cell responses only in IgE^+ subjects show higher 3D surface accessibility of the peptide center than the ends. These trends reveal promising predictors to further improve the *in silico* prediction of allergenicity of proteins.

III-666

Detection of reactive chemicals and oxidants using an organotypic human airway model with Nrf2 reporter activity (EpiAirway-Nrf2): Application to evaluation of tobacco products

Patrick Hayden, George R. Jackson, Steven Coughlin, Olivia O'Connell, Anna Maione and Mitchell Klausner

MatTek Corporation, Ashland, MA, United States
phayden@mattek.com

The Nrf2 transcription factor controls expression of enzymes involved in defense against electrophilic and oxidative damage. We developed an organotypic model of human airway epithelium (EpiAirway-Nrf2) containing an Nrf2 luciferase reporter for use in toxicity screening of inhaled chemicals. The model was characterized with 12 reference chemicals, whole tobacco smoke and e-cigarette vapor. Strongly electrophilic chemicals known to form covalent adducts with cellular biomolecules (acrolein, iodoacetamide, nitrobenzylbromide, cinnamaldehyde, dinitrochlorobenze, t-butylhydroquinone) elicited strong induction of Nrf2. Whole tobacco smoke also induced strong Nrf2 activation. E-cigarette vapor produced only weak activation. The results demonstrate that the Nrf2 airway reporter model is a highly sensitive detector of reactive electrophilic chemicals or mixtures including whole tobacco smoke. The model may prove useful for safety evaluation of new generation nicotine delivery products.

III-677

Optimization of airway epithelium organotypic culture models as a platform for adverse outcome pathway assessment of engineered nanomaterials

Tyler Nicholas[1], Anoria Haick[2], Terrance Kavanagh[1], Elaine Faustman[1] and William Altemeier[2]

[1]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States; [2]Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA, United States
nicholat@uw.edu

Engineered nanomaterials (ENM), including silver nanoparticles (AgNP), are one of the largest groups of emerging potential toxicants. In this study, we are developing airway epithelium organotypic culture models (AE-OCM) as a platform to predict pathways of response to AgNP. We studied response to AgNP in AE-OCM undifferentiated and differentiated under normal and IL-13 conditions to reflect airway epithelium of "proliferating", "healthy", and "disease" phenotypes, respectively. We quantified differences in: cytotoxicity, antioxidant and unfolded protein response, and chemokine release. In normal differentiated AE-OCM derived from A/J and C57BL/6J mice, cytotoxicity increased from 5.0% to 15.5% and 3.7% to 28.1%, respectively. In IL-13 differentiated AE-OCM derived from A/J and C57BL/6J mice, cytotoxicity increased from 4.2% to 23.4% and 5.2% to 26.9%, respectively. This study provides new information on potency and adverse outcome pathways for these critical endpoints of respiratory toxicity.

III-702

Establishment of the new *in vitro* nasal epithelial model (SoluAir™) for alternative toxicity test

Su Hyon Lee, Seol Yeong Kim and Hye Soo Kim

Biosolution Inc., Seoul, South Korea
lulublue@hanmail.net

Human 3D airway model is promising model for safety and efficacy evaluation of compounds targeting the airway. Therefore, the present study attempted to establish the new *in-vitro* nasal epithelial model (SoluAir™) for alternative toxicity test. Human nasal epithelial (HNE) cells were seeded onto 12 mm millicell, cultured for 7 days of submerged condition and following 7 days of air-liquid interface condition. Fully differentiated cell sheets were compared with intact human nasal epithelium by H&E and immunohistochemical staining. We confirmed primary cultured HNE cells showed cobblestone-like morphology and well differentiated ciliogenesis. Furthermore, after long term period (30 days of ALI condition), SoluAir™ showed an increased cilia and maturation of goblet cells. These results suggest that SoluAir™ is suitable for alternative toxicity test owing to its similarity with nasal epithelium.

References

Million, K., Tournier, F., Houcine, O. et al. (2001). Effects of retinoic acid receptor-selective agonist on human nasal epithelial cell differentiation. *Am J Respir Cell Mol Biol 25*, 744.

Saim, A. B., Chua, K. H. and Idrus, R. (2007). Human nasal turbinates as a viable source of respiratory epithelial cells using co-culture system versus dispase dissociation technique. *Laryngoscope 117*, 2139.

Schmidt, D., Hubsch, U., Wurzer, H. et al. (1996). Development of an in vitro human nasal epithelial (HNE) cell model. *Toxicol Lett 88*, 75.

Woodworth, B. A., Antunes, M. B., Bhargave, G. et al. (2007). Murine tracheal and nasal septal epithelium for air-liquid interface cultures: A comparative study. *Am J Rhiol 21*, 533.

Yeh, T. H., Tsai, C. H., Chen, Y. S. et al. (2007). Increased communication among nasal epithelial cells in air-liquid interface culture. *Laryngoscope 117*, 1439.

Innovative Models for Safety and Efficacy - Developmental Neurotoxicity

III-155

Leveraging high-content screening in zebrafish embryos to identify anemia-inducing chemicals

David Volz

University of California, Riverside, Riverside, CA, United States
david.volz@ucr.edu

Using high-content screening, we recently identified butafenacil as a potent inducer of anemia in zebrafish embryos. Butafenacil is an herbicide that inhibits protoporphyrinogen oxidase (PPOX) – an enzyme that catalyzes oxidation of protoporphyrinogen IX to protoporphyrin IX during chlorophyll and heme biosynthesis. We then revealed that severe butafenacil-induced anemia in zebrafish was due to a loss of hemoglobin. In addition, while the magnitude of butafenacil-induced anemia was similar in the presence or absence of light, protoporphyrin accumulation and acute toxicity were significantly lower or absent when embryos were exposed under dark conditions. Finally, we found that butafenacil would not have been prioritized by the US EPA's ToxCast program due to the absence of ToxCast assays that identify PPOX inhibitors and/or chemicals affecting hematopoiesis. Overall, this study highlights the potential utility of our assay for identifying anemia-inducing chemicals.

III-261

Methylglyoxal: Acute toxicity evaluation in co-culture model of human neurons and astrocytes

Francesca Caloni[1], Uliana De Simone[2], Adela M Pintea[3], Giorgio Marrubini[4], Adele Papetti[4] and Teresa Coccini[2]

[1]Dipartimento di Medicina Veterinaria (DIMEVET), Università degli Studi di Milano, Milan, Italy; [2]Laboratory of Clinical and Experimental Toxicology, Toxicology Unit, Maugeri Clinical Scientific Institutes SpA-BS, IRCCS, Pavia, Italy; [3]Department Preclinical Sciences, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; [4]Department of Drug Sciences, University of Pavia, Pavia, Italy
francesca.caloni@unimi.it

Methylglyoxal (MG) is a major cell-permeant precursor of advanced glycation end-products which are implicated in various pathophysiological mechanisms including neurodegenerative diseases. Acute (24-48 h) toxicity effects of MG (0.5-1 mM) were assessed on human astrocytes (D384) and neurons (SH-SY5Y) co-cultures and compared to those obtained in the respective mono-cultures. MG caused marked metabolic changes (MTT) and morphological alterations (contrast-phase microscopy) in monocultured neurons. However, SH-SY5Y cell viability was significantly less affected, by MG exposure, when co-cultured with astrocytes. Similarly to neurons, D384 cells in coculture system tolerated more MG than in monoculture.

In summary, astrocytes, when cultured together with neurons, diminished the neurotoxicant-induced cytotoxic effects occurred in neurons cultured under solitary conditions, and become themselves more resistant in the presence of neurons.

Grant: Italian Ministries of Health, Research & Education.

III-271

Advanced *in vitro* models for assessing neurotoxicity of Fe_3O_4 nanoparticles: Co-culture models and 3D spheroid cultures of human origin

Francesca Caloni[1], Uliana De Simone[2], Arsenio Spinillo[3], Marianna Roccio[3], Maria Antonietta Avanzini[4] and Teresa Coccini[2]

[1]Dipartimento di Medicina Veterinaria (DIMEVET), Università degli Studi di Milano, Milan, Italy; [2]Maugeri Clinical Scientific Institutes SpA-BS, IRCCS, Pavia, Italy; [3]Department of Obstetrics and Gynecology, IRCCS Foundation Policlinico San Matteo and University of Pavia, Pavia, Italy; [4]Laboratory of Transplant Immunology/Cell Factory, IRCCS Foundation Policlinico San Matteo, Pavia, Italy
francesca.caloni@unimi.it

New *in vitro* models namely (i) 2D co-cultures (i.e., human SH-SY5Y neuroblastoma and D384 astrocytoma cells, using a transwell system), and (ii) 3D spheroid cultures (using ultra-low attachment 96-well round bottomed plates) of SH-SY5Y and D384, were applied to evaluate toxicity (mitochondrial activity and morphology effects) after short-term exposure to Fe_3O_4 nanoparticles (NP) (1-100 mg/ml) which have attracted extensive interest in biomedical and industrial fields.

Astrocytes, when co-cultured with neurons, attenuated the NP-induced cytotoxic effects occurred in neurons grown under solitary conditions after 48 h, and become themselves more resilient in the presence of neurons. Notably, when using 3D spheroid models, exhibiting features that are closer to the complex *in vivo* conditions, a significantly additional reduction of NP-induced toxicity was detected in both SH-SY5Y and D384 compared to effects observed in co-cultures.

Grant "5x1000 project 2011" Italian Ministry of Health.

Reference

Coccini, T., Caloni, F., Ramírez Cando, L. J. and De Simone, U. (2017). Cytotoxicity and proliferative capacity impairment induced on human brain cell cultures after short- and long-term exposure to magnetite nanoparticles. *J Appl Toxicol 37*, 361-373. doi:10.1002/jat.3367

III-294

Zebrafish model as a tool for evaluating autistic effects: A preliminary study

Sangwoo Lee, Hang-Suk Chun, Woo-Keun Kim and Seokjoo Yoon

System Toxicology Research Center, Korea Institute of Toxicology, Daejeon, South Korea
sangwoo.lee@kitox.re.kr

The autism prevalence has recently been increased. However, most researches are limited to human and rodents model. In this study, to develop a new model for evaluating autism, early developmental stage zebrafish were employed. We observed behavioral and developmental changes and conducted transcriptome analysis using a RNA-seq after exposure to valproic acid (VPA), an anti-epileptic drug and known as an autism inducer. Hatching rate and time to hatch was significantly altered at 100 μM and 50 μM VPA, respectively. Total travelled distance and actively moved duration was decreased at 50 μM VPA. In transcriptomic analysis, genes related to neurogenesis, nervous system development and sensory perception were significantly affected. Our data showed that the effects on zebrafish embryo could be linked to autism. As a preliminary study, our study could contribute to developing zebrafish model for evaluating autism and to improving current understanding of molecular mechanism of VPA.

References

Bailey, J. M., Oliveri, A. N., Karbhari, N. et al. (2016). Persistent behavioral effects following early life exposure to retinoic acid or valproic acid in zebrafish. *Neurotoxicology 52*, 23-33.

Chang, J., Gilman, S. R., Chiang, A. H. et al. (2015). Genotype to phenotype relationships in autism spectrum disorders. *Nat Neurosci 18*, 191-198.

Zimmermann, F. F., Gaspary, K. V., Leite, C. E. et al. (2015). Embryological exposure to valproic acid induces social interaction deficits in zebrafish (Danio rerio): A developmental behavior analysis. *Neurotoxicol Teratol 52*, 36-41.

III-539

Triclosan exposure is toxic to neuronal maturation stage in zebrafish embryo neurodevelopment

Jin Kim[1], C-yoon Kim[1], Hanseul Oh[1], Bokyeong Ryu[1], Ukjin Kim[1], Ji Min Lee[1], Cho-Rok Jung[2] and Jae-Hak Park[1]

[1]Seoul National University, Seoul, South Korea; [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea
jk208019@snu.ac.kr

Triclosan (TCS) is a compound with a wide range of antibiotic activity and has been widely used. It has recently been shown to reduce viability and activate apoptotic pathways in neuronal cells. However, there is relatively little research on the mechanism of neurotoxicity *in vivo*. In this study, we performed morphological analysis, fluorescence analysis using transgenic zebrafish, and expression analysis of genes to clarify the effect of TCS on neurogenesis and apoptosis-related changes. TCS decreased the body length, head size, and eye size in a concentration-dependent manner and affected the CNS fluorescence structure, resulting in decreased synaptic formation and shorter axon length. In addition, it significantly altered the expression of the neurogenic genes and increased the apoptosis related cells and genes. Our study demonstrates *in vivo* that triclosan is toxic in certain neurogenesis stages, particularly neuronal maturation, and induces apoptosis.

References

Park, B. K., Gonzales, E. L., Yang, S. M. et al. (2016). Effects of triclosan on neural stem cell viability and survival. *Biomol Ther 24*, 99-107.

Ruszkiewicz, J. A., Li, S., Rodriguez, M. B. and Aschner, M. (2017). Is triclosan a neurotoxic agent? *J Toxicol Environ Health B Crit Rev 20*, 104-117.

Szychowski, K. A., Sitarz, A. M. and Wojtowicz, A. K. (2015). Triclosan induces Fas receptor-dependent apoptosis in mouse neocortical neurons in vitro. *Neuroscience 284*, 192-201.

III-599

The role of autism risk gene, CHD8, in chlorpyrifos-induced neurotoxicity in iPSC-derived brain organoids

Xiali Zhong[1,2], David Pamies[3], Helena Hogberg[3], Herbert M. Lachman[4], Thomas Hartung[3] and Lena Smirnova[3]

[1]Environmental Health Science, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States; [2]Health Toxicology, School of Public Health, Central South University, Changsha, People's Republic of China; [3]Center for Alternatives to Animal Testing (CAAT), Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States; [4]Department of Genetics, Albert Einstein College of Medicine, Brons, NY, United States
xzhong16@jhu.edu

To investigate gene environment interactions, contributing to autism, we established human iPSC-derived 3D brain model from heterozygous ($CHD8^{+/-}$) knockout iPS cells along with wild-type control cell line ($CHD8^{+/+}$). CHD8 is one of autism risk genes. At 4 weeks of differentiation, cell viability, mitochondrial activity and the neural migration were reduced in dose dependent manner after 24 h exposure to CPF. Those effects were stronger in $CHD8^{+/-}$ cell line vs. control. CPF elevated *FOXG1* gene and expression of autism associated gene, *AUTS2*, in both cell lines. Then, loss of CHD8 caused stronger effects of CPF on synaptic formation: stronger inhibition of acetylcholinesterase activity; decreased levels of synaptophysin/PSD95 and repression of genes involved in cell-to-cell communication (*GRIN1*, *NRXN2*, *SHANK3*, *NLGN3*). These results suggest that genetic mutation and environmental factor may interplay in the neurodevelopmental perturbations, such as autism.

III-637

iPSC and 3D tissue technologies – Powerful alternatives to animal models for brain disease research

Marcia Triunfol[1] and Catherine Willett[2]

[1]Humane Society International, Rio De Janeiro, Brazil; [2]Regulatory Toxicology, Risk Assessment and Alternatives, The Humane Society of the United States, Gaithersburg, MD, United States
mtriunfol@hsi.org

The average cost of research and development for a drug is about US$2.6 billion and more than 90% of compounds entering clinical trials fail, mainly as a result of lack of efficacy and/or unacceptable toxicity due in large part to the lack of translation from preclinical animal test result to humans. Yet medical research remains heavily invested in non-human animals, especially rodents. However, a new scenario is emerging in Brazil, where the Zika outbreak has pushed local scientists to find answers fast using innovative technology that in most cases does not rely on animals. This scenario has created a fertile environment to discuss the potential of 3D tissue and iPSC technology as viable, safe, powerful and cost-effective alternatives to failing animal models. Here we present the conclusions and recommendations of a 2-day meeting that gathered Brazilian and International scientists working on a range of brain diseases, together with investors, policy makers, and regulators.

III-690

Development of a dysmyelination test to study developmental neurotoxicity of environmental chemicals in an iPSC-derived human brain microphysiological system

Megan Chesnut, Helena Hogberg, Thomas Hartung and David Pamies

Center for Alternatives to Animal Testing, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States
mchesnu1@jhu.edu

Neurodevelopment is highly sensitive to perturbations caused by environmental exposure, and myelin formation is a key neurodevelopmental event. Chemical disruption of myelin formation has been demonstrated in animal developmental neurotoxicity (DNT) studies, but very few *in vitro* human cell-based models of myelination exist. Our center has recently developed a reproducible human iPSC-derived brain microphysiological system (BMPS) that models key neurodevelopmental events, and wrapping of axonal structures by oligodendrocyte processes was confirmed with confocal and electron microscopy (Pamies et al., 2016). The objective of this study is to investigate whether chemicals that disrupt myelination during neurodevelopment can be identified with our *in vitro* system. Using myelin basic protein immunostaining and computer-assisted evaluation of myelin formation (Kerman et al., 2015), we have been able to quantify axon myelination in BMPS. Preliminary data has demonstrated that the organophosphate flame retardant TDCPP causes dose-dependent reductions in myelin formation in BMPS, indicating that the BMPS has potential as a novel DNT test system to study dysmyelination.

References

Kerman, B. E., Kim, H. J., Padmanabhan, K. et al. (2015). In vitro myelin formation using embryonic stem cells. *Development 142*, 2213-2225.

Pamies, D., Barreras, P., Block, K. et al. (2016). A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity. *ALTEX 34*, 362-376.

Innovative Models for Safety and Efficacy – Skin

III-16

Comparative study on ARE-Nrf2 luciferase assay, spectrophotometric direct peptide reactivity assay and the human cell activation test according to the lipophilicity

Sun A. Cho[1], Minseok Choi[1], Sae-Ra Park[1], John Hwan Lee[1], Bae Hwan Kim[2] and Susun An[1]

[1]Amorepacific R&D Unit, Seoul, South Korea; [2]Keimyung University, Daegu, South Korea

sunacho@amorepacific.com

Since the animal testing has been ban, cosmetics industry has need animal alternative skin sensitization test method that shows high accuracy. In addition, it is also important to choose the test methods having wide coverage of test material. For example, because lots of cosmetics ingredients are lipophilic, many alternative test methods have limitation to test these materials. In this study, we performed the comparative study using ARE-Nrf2 luciferase assay, h-CLAT (human cell activation test) and Spectrophotometric DPRA (direct peptide reactivity) for chemicals that has wide range of lipophilicity. In case of high lipophilic chemical (above log P 7.0), test couldn't be carried out because of limitation of dispersion in h-CLAT, however ARE-Nrf2 luciferase assay and Spectrophotometric DPRA could be performed. From these result, ARE-Nrf2 luciferase assay and Spectrophotometric DPRA could be a more useful method to estimate the skin sensitization of lipophilic cosmetic ingredients.

III-43

Cosmetics Europe assessment of non-animal approaches for predicting skin sensitization

Martina Klaric[1], Nathalie Alépée[2], Dave Allen[3], Takao Ashikaga[4], Warren Casey[5], Elodie Clouet[6], Magalie Cluzel[7], Aurelia Del Bufalo[2], Nikki Gellatly[8], Carsten Goebel[9], Sebastian Hoffmann[10], Petra Kern[11], Nicole Kleinstreuer[5], Jochen Kuehnl[12], Karsten Mewes[13], Masaaki Miyazawa[14], Dirk Petersohn[13], Judy Strickland[3], Erwin Van Vliet[15] and Dan Zang[3]

[1]Cosmetics Europe, Brussels, Belgium; [2]L'Oréal, Aulnay sous Bois, France; [3]Integrated Laboratory Systems, Inc, Research Triangle Park, NC, United States; [4]Shiseido, Kanagawa, Japan; [5]NICEATM, Research Triangle Park, NC, United States; [6]Pierre Fabre, Castres, France; [7]LVMH, St Jean de Braye, France; [8]Unilever, Bedford, United Kingdom; [9]Coty, Darmstadt, Germany; [10]seh consulting + services, Paderborn, Germany; [11]Procter & Gamble, Strombeek-Bever, Belgium; [12]Beiersdorf AG, Hamburg, Germany; [13]Henkel AG, Düsseldorf, Germany; [14]Kao, Tochigi, Japan; [15]SeCAM, Magliaso, Switzerland

mklaric@cosmeticseurope.eu

Skin sensitization is a toxicity endpoint of widespread concern, for which non-animal testing approaches are available. Cosmetics Europe and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods analyzed the performance of multiple non-animal data integration approaches for the skin sensitization safety assessment. We collected and generated data on 128 substances in multiple *in vitro* and *in chemico* skin sensitization assays which are key components of various non-animal defined approaches to testing and assessment that have been submitted to the OECD as case studies for skin sensitization. LLNA and human sensitization data were used to evaluate the performance of multiple non-animal testing strategies for hazard and potency characterization. Defined approaches examined include consensus methods, artificial neural networks, support vector machine models and decision tree, all of which were reproduced using open source software tools.

III-44

The validation study for condition setting of DPRA method in Korea

Bae-Hwan Kim and Kyo-Hyun Park

Keimyung University, Daegu, South Korea

kim9399@kmu.ac.kr

The purpose of this study is to condition setting of DPRA (direct peptide reactivity assay) that is alternative method to skin sensitization test throughout the validation study in Korea. We conducted intra-lab (triplicate) and inter-lab (3 labs, CRI, KTR, CU) validation using 20 chemicals (10 chemicals for the proficiency test listed in OECD guideline 442C and additional 10 more chemicals by reference papers). All data of 3 labs were met with acceptance criteria. In intra-lab validation, two of 12 positive chemicals showed false negative (sensitivity: 83%), and one of 8 negative chemicals showed false positive (specificity: 88%). In inter-lab validation, the accuracy of 3 labs were equal to 90% (Data of sensitivity and specificity were 83 and 100% for 2 labs, and 92 and 88% for 1 lab). During these studies, we also checked and improved various limitations that may arise. These data indicated that DPRA method proposed by OECD TG442C is expected to be possibly well established in Korean GLP applied system.

References

Cho, S. A., Jeong, Y. H., Kim, J. H. et al. (2014). Method for detecting the reactivity of chemicals towards peptides as an alternative test method for assessing skin sensitization potential. *Toxicol Lett 225*, 185-191.

Dimitrov, S., Detroyer, A., Piroird, C. et al. (2016). Accounting for data variability, a key factor in in vivo/in vitro relationships: Application to the skin sensitization potency (in vivo LLNA versus in vitro DPRA) example. *J Appl Toxicol 36*, 1568-1578. doi:10.1002/jat.3318

OECD (2015). Test Guideline 442C: In chemico skin sensitization: Direct Peptide Reactivity Assay (DPRA).

Takenouchi, O., Fukui, S., Okamoto, K. et al. (2015). Test battery with the human cell line activation test, direct peptide reactivity assay and DEREK based on a 139 chemical data set for predicting skin sensitizing potential and potency of chemicals. *J Appl Toxicol 35*, 1318-1332. doi:10.1002/jat.3127

Urbisch, D., Honarvar, N., Kolle, S. N. et al. (2016). Peptide reactivity associated with skin sensitization: The QSAR Toolbox and TIMES compared to the DPRA. *Toxicol In Vitro 34*, 194-203. doi:10.1016/j.tiv.2016.04.005

III-49

The long form of thymic stromal lymphopoietin of keratinocytes was induced by protein allergens via the NF-κB signaling pathway

Yasutaka Kuroda[1,2], Takuo Yuki[1], Yutaka Takahashi[1], Hitoshi Sakaguchi[1], Kayoko Matsunaga[3] and Hiroshi Itagaki[2]

[1]Kao Corporation, Tochigi, Japan; [2]Yokohama National University, Kanagawa, Japan; [3]Fujita Health University School of Medicine, Aichi, Japan

kuroda.yasutaka@kao.co.jp

Epicutaneous sensitization of protein allergens induces immediate-type hypersensitivity following type 2 immune response development. Keratinocyte-derived thymic stromal lymphopoietin (TSLP) activates dendritic cells and induces type 2 immune responses. Previously, we demonstrated that protein allergens in cultured human keratinocytes strongly induced long form TSLP (loTSLP) transcription. This study aimed to evaluate the response of TSLP to complex or simple protein allergen components and to identify regulated factors. LoTSLP mRNA was strongly induced by complex allergens (naïve and acid-hydrolyzed wheat gluten) and allergen components (ovalbumin, trypsin, and hev b 1) but not by human serum albumin and hev b 6.02. Allergen-induced loTSLP mRNA was significantly suppressed by nuclear factor-κB inhibitors (parthenolide, ammonium pyrrolidinedithiocarbamate, JSH-23). Thus, we suggest that some allergen components induced intense loTSLP transcription via nuclear factor-κB signaling.

III-51

Multi-Immuno Tox Assay (MITA): The creation of its data set and the results of validation studies

Yutaka Kimura[1], Rie Yasuno[2], Mika Watanabe[3], Miwako Kobayashi[3], Tomoko Iwaki[4], Chizu Fujimura[1], Yoshihiro Ohmiya[2], Kohji Yamakage[3], Yoshihiro Nakajima[4], Mayumi Kobayashi[5], Takashi Omori[5], Hajime Kojima[6] and Setsuya Aiba[1]

[1]Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan; [3]Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan; [4]Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan; [5]Division of Biostatistics, Department of Social/Community Medicine and Health Science, Kobe University School of Medicine, Kobe, Japan; [6]Japanese Center for the Validation of Alternative Methods, National Institute of Health Sciences, Tokyo, Japan

yutakimu@mte.biglobe.ne.jp

We established a luciferase reporter assay system, Multi-Immuno Tox Assay (MITA), which are composed of an IL-2/IFN-γ reporter cell, #2H4, an IL-8 reporter cell, THP-G8, and an IL-1β reporter cell, THP-G1b, to evaluate the effects on key predictive *in vitro* components of the human immune system. We first performed a validation study of MITA using #2H4 to examine the transferability and within- and between-laboratory reproducibility in three independent laboratories (Phase I). We assessed 5 coded chemicals 3 times. The results demonstrated that within-laboratory and between-laboratory reproducibility are 86.7% (13/15) and 80.0% (4/5), respectively. In addition, we created a dataset on 60 chemicals based on the previously reported data set, and then, demonstrated that immunotoxicants can be divided into four categories, IL-2 transcript suppressor, sensitizer, IL-8 transcript suppressor and IL-2 transcript stimulator.

Reference

Kimura, Y. et al. (2014). Evaluation of the multi-immunotox assay composed of 3 human cytokine reporter cells by examining immunological effects of drugs. *Toxicol In Vitro 28*, 759-768.

III-68

Getting under the skin of an *in silico* approach to predicting dermal sensitisation

Donna Macmillan and Martyn Chilton

Lhasa Limited, Leeds, United Kingdom

donna.macmillan@lhasalimited.org

In silico models have emerged as a popular non-animal method to predict skin sensitisation, due in part, to worldwide pressure to reduce, refine and replace the use of animals in science.

Derek Nexus (http://www.lhasalimited.org) is a toxicity prediction tool which can predict skin sensitisation hazard (sensitiser/non-sensitiser) with an accuracy of 75%, a positive predictivity of 73% and a negative predictivity of 76% (n = 2571). A more challenging aspect for *in silico* tools is quantification of risk and a concerted effort to tackle this is on-going. By using mechanism based structural alerts (based on both public and proprietary toxicity data) to provide quantitative predictions of the local lymph node assay (Canipa et al., 2017) a measure of risk (predicted EC3) can now be estimated – and enhanced when subject to expert review. Additionally, *in silico* predictions can also be used in an integrated testing strategy alongside *in vitro* and/or *in chemico* assays to improve predictivity when compared to assays alone (Macmillan et al., 2016).

This multifaceted *in silico* approach to predicting skin sensitisation hazard and risk is evaluated using a number of relevant case studies to illustrate the benefits and drawbacks of each element.

References

Canipa, S. J., Chilton, M. L., Hemingway, R. et al. (2017). A quantitative in silico model for predicting skin sensitisation using a Nearest Neighbours approach within expert-derived structure-activity alert spaces. *J Appl Toxicol 37*, 985-995.

Macmillan, D. S., Canipa, S. J. and Chilton, M. L. (2016). Predicting skin sensitisation using a decision tree integrated testing strategy with an in silico model and in chemico/in vitro assays. *Regul Toxicol Pharmacol 76*, 30-38.

III-71

Expression of microRNAs in a myeloid cell line stimulated with sensitizers and allergens formed during rubber vulcanization

Tim Lindberg[1], Kathrin Zeller[1], Andy Forreryd[1], Rolf Søkilde[2], Carlos Rovira[2], Ola Bergendorff[3] and Malin Lindstedt[1]

[1]Department of Immunotechnology, Lund, Sweden; [2]Oncology and Pathology, Faculty of Medicine, Lund, Sweden; [3]Occupational and Environmental Dermatology, Faculty of Medicine, Malmö, Sweden
tim.lindberg@immun.lth.se

Repeated skin exposure to sensitizing chemicals can induce an immunological response in susceptible individuals causing allergic contact dermatitis (ACD). Such chemicals are used in everyday products and can even lead to ACD in certain occupational groups, e.g. health care professions using rubber gloves. Methods to determine the hazard of chemicals exists but the molecular mechanisms underlying sensitization are still not completely understood. In this study, we investigated the role of microRNAs in sensitization by profiling the expression of 800 microRNAs in a myeloid cell line stimulated with sensitizing chemicals, including three rubber allergens, and controls. Several microRNAs were differentially regulated by the sensitizing chemicals as a group, while some were regulated in response to a unique chemical. These data together with appropriate functional analyses can give valuable insight about the sensitization reaction resulting in better outlooks for chemical risk assessment.

References

Ezendam, J., Braakhuis, H. M. and Vandebriel, R. J. (2016). State of the art in non-animal approaches for skin sensitization testing: From individual test methods towards testing strategies. *Arch Toxicol 90*, 2861-2883, doi:10.1007/s00204-016-1842-4

Geier, J., Lessmann, H., Uter, W. and Schuch, A. (2003). Occupational rubber glove allergy: Results of the Information Network of Departments of Dermatology (IVDK), 1995-2001. *Contact Dermatitis 48*, 39-44.

Kimber, I., Basketter, D. A., Gerberick, G. F. and Dearman, R. J. (2002). Allergic contact dermatitis. Int Immunopharmacol 2, 201-211.

Kohl, L., Blondeel, A. and Song, M. (2002). Allergic contact dermatitis from cosmetics. Retrospective analysis of 819 patch-tested patients. *Dermatology 204*, 334-337.

III-86

Using the co-culture system to assess chemicals for skin sensitization

Yu Chen[1], Yihui Ke[2], Jian Chen[2] and Zhijie Chen[2]

[1]Guandong Inspection and Quarantine Technology Center, Pearl River New Town, Guangzhou, People's Republic of China; [2]Guangzhou CHN-ALT Biotechnology Ltd, Guangzhou, People's Republic of China
cy94776@163.com

The human cell line activation test (h-CLAT) was published in OECD TG442E for assessment of skin sensitization. The h-CLAT was just contained a kind of dendritic cell (THP-1 cells), which is a key event of Adverse Outcome Pathways (AOP). As well as the Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitization was constructed, for example, a series of test methods in OECD TG442B to 442E, Bayesian integrated testing strategy, artificial neural network model, etc.

We have developed two methods, which were the HaCaT cell and a 3D reconstructed epidermis (Episkin™) cultured with THP-1 cell, respectively. The HaCaT cell was combined with THP-1 cell by co-culture system, which provide three index included IL-18 from HaCaT cell and CD86/CD54 surface markers. However, the system used the epidermis overly the THP-1 cells was built successfully, and it was analyzed the transcriptome of the cells from epidermis and the CD86/CD54 surface markers from THP-1 cells. These co-culture systems are under validation.

III-90

Simultaneous performance of skin allergy tests in a 2-Organ-Chip (2-OC) platform

Eduardo Pagani[1], Fernanda Luisa Basei[1], Talita Miguel Marin[1], Desiree Cigaran Schuck[2], Carla Abdo Brohem[2], Marcio Lorencini[3] and Alisson Campos Cardoso[1]

[1]LNBio/CNPEM, RENAMA, Campinas, Brazil; [2]Center for Safety and Efficacy Assessment, Grupo Boticário, São José dos Pinhais, Brazil; [3]Products Evaluation and Regulatory Affairs, Grupo Boticário, São José dos Pinhais, Brazil
eduardo.pagani@lnbio.cnpem.br

Microfluidic chips are promising for developing more predictive non-animal tests. This study aims at the simultaneous assessment of 2 key events of Skin Sensitization: the inflammatory response in keratinocytes and the activation of dendritic cells. Reconstituted human skin and human monocytic leukemia cell line (THP-1) were simultaneously cultivated in different compartments of a microfluidic chip (2-OC by TissUse GmbH). The drug 2,4-dinitrochlorobenzene (DNCB) was placed on the skin or in the culture media for 48 hours. Some adaptations were made to the OECD guidelines for optimized transposition from separated static tests to their simultaneous performance in the 2-OC. The activation of the Keap1-Nrf2-ARE pathway in the skin (qPCR) and the CD86 expression in THP-1 cells (flow cytometry) were bigger when the DNCB was placed on the skin. These results show that the 2-OC can emulate better the real allergenic process and has potential to improve the accuracy of *in vitro* skin allergy tests.

References

Gordon, S., Daneshian, M. and Bouwstra, J. (2015). Non-animal models of epithelial barriers (skin, intestine and lung) in research, industrial applications and regulatory toxicology. *ALTEX 32*, 327-378. doi:10.14573/altex.1510051

OECD Test No. 442D: In vitro Skin Sensitisation, ARE-Nrf2 Luciferase Test Method.

OECD Test No. 442E: In vitro Skin Sensitisation, Human Cell Line Activation Test (h-CLAT).

III-124

An innervated and vascularized immunocompetent tissue-engineered skin to study cutaneous neuroinflammation

Quentin Muller[1,2], Marie-Josée Beudet[1], Evelyne Schaeffer[1,2], François Berthod[1] and Vincent Flacher[2]

[1]Department of Surgery, LOEX, Québec City, QC, Canada; [2]CNRS-UPR3572, IBMC, Strasbourg, France

q.muller@ibmc-cnrs.unistra.fr

Immune reactions in the skin are initiated by the cutaneous dendritic cells (DCs) (Schaeffer et al., 2014). The potential sensitizing effect of a compound can be predicted *in vitro* using human monocytes differentiated into DCs (Mono-DCs). However, the accuracy of this approach remains limited because the activation of cutaneous DCs by sensitizers may be elicited after crossing the epidermal barrier and interacting with nerves and the cutaneous microenvironment (Cadau et al., 2015). Our goal was to develop an immunocompetent tissue-engineered skin that combine a functional epidermis and a network of endothelial capillaries and nociceptive nerve fibers. Our model was seeded with fibroblasts and endothelial cells, with either human iPS cell-derived or murine embryonic neurons, keratinocytes and Mono-DCs. This model will be used to predict the irritant potential of chemical compounds, and the impact of nerves on DC activation.

References

Cadau, S., Leoty-Okombi, S., Pain, S. et al. (2015). In vitro glycation of an endothelialized and innervated tissue-engineered skin to screen anti-AGE molecules. *Biomaterials 51*, 216-225.

Schaeffer, E., Flacher, V., Papageorgiou, V. et al. (2014). Dermal Cd14 dendritic cell and macrophage infection by dengue virus is stimulated by interleukin-4. *J Invest Dermatol 135*, 1743-1751.

III-127

Performance of the GARD Assay in a blinded Cosmetics Europe study

Peter Sandberg[1], Henrik Johansson[1], Robin Gradin[1], Andy Forreryd[2], Maria Agemark[1], Kathrin S. Zeller[2], Anki Malmborg Hager[1], Angelica Johansson[1], Olivia Larne[1], Erwin van Vliet[3], Carl Borrebaeck[2] and Malin Lindstedt[2]

[1]SenzaGen, Lund, Sweden; [2]Immunotechnology, Lund University, Lund, Sweden; [3]Cosmetics Europe, Brussels, Belgium

henrik.johansson@senzagen.com

To mitigate the risk of allergic contact dermatitis in relation to the use of consumer products, powerful tools to test chemicals are vital. The GARDskin (Genomic Allergen Rapid Detection) (Johansson et al., 2013) address this question and was developed to meet the demand of non-animal methods. Here, we reconfirm the performance and accuracy of GARDskin. A human myeloid cell line was separately stimulated with 72 blinded chemicals, provided by Cosmetics Europe. Post incubation the gene expression of 200 predictive genomic biomarkers were quantified. The high-dimensional data was analyzed by machine learning techniques and each chemical was classified as sensitizer or non-sensitizer. Hereby, the predictive accuracy of GARD compared to a composite reference of human and local lymph node assay data (Basketter et al., 2014; CE database) was calculated to 83% in this blinded dataset. By combining previous GARD datasets with the novel, the accuracy reaches 86% (127 chemicals). This demonstrates the functionality of an advanced genomic test strategy.

References

Basketter, D. A., Gerberick, G. F. and Kimber, I. (2014). The local lymph node assay in 2014. *Dermatitis 25*, 49-50.

Johansson, H., Albrekt, A. S., Borrebaeck, C. A. and Lindstedt, M. (2013). *Toxicol In Vitro 27*, 1163-1169.

III-142

The cross talk between antigen-presenting cells and epithelial cells *in vitro* at transcriptome level

Mario T. Schellenberger[1], Sébastien Cambier[2], Jennifer Hennen[1], Vladimir Benes[3], Tommaso Serchi[2], Arno C Gutleb[2] and Brunhilde Blömeke[1]

[1]Department of Environmental Toxicology, Trier University, Trier, Germany; [2]Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology, Esch-sur-Alzette, Luxembourg; [3]GeneCore Genomics Core Facilities, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

schellenberger@uni-trier.de

We established a coculture assay for the *in vitro* prediction of sensitizing chemicals composed of THP-1 cells, as model for dendritic cells and HaCaT keratinocytes. In this study, we further elucidate the cross talk in our coculture assay analyzing the transcriptome of THP-1 cells. After coculture, THP-1 cells were harvested and cells were analyzed by NGS and gene expression was compared to monocultured THP-1 cells. Gene ontology enrichment analysis showed that the 585 differentially expressed genes are involved in GM-CSF-, IL-3-, IL-5-mediated signaling, β1-integrin cell surface interactions and INF-γ pathway. Up to now it is not clear whether the cross talk between HaCaT and THP-1 cells comprises the exchange of mRNA or whether it influences the gene expression in THP-1 cells, or both. These results strengthen the hypothesis that cross talk is taking place between the two cell lines and may help to improve *in vitro* prediction of sensitizing potential and potency of chemicals.

III-152

Photoprotective potential and phototoxicity of fucoxanthin: A new UV-filter candidate based on Antarctic brown algae

Renata Spagolla Napoleão Tavares[1], Gabriela Timotheo do Amaral[1], Carolina Gomes Benevenuto[1], Silvya Stuchi Maria-Engler[2], Pio Colepicolo Neto[3], Hosana Maria Debonsi[4] and Lorena Rigo Gaspar Cordeiro[1]

[1]Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences at Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; [2]Clinical Chemistry and Toxicology, University of São Paulo, São Paulo, Brazil; [3]Chemistry Institute, University of São Paulo, São Paulo, Brazil; [4]Physics and Chemistry, Faculty of Pharmaceutical Sciences at Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

renatasnt@usp.br

The discovery of new biological UVA filter amplifies the range of options for sunscreens. The main marine carotenoid, fucoxanthin, involved on photoprotection of brown algae, was isolated from an Antarctic Desmarestia anceps. The aim of this study was to evaluate the photoprotective potential and its phototoxicity. This substance was submitted to analysis of the UV spectra, photostability, phototoxicity by using 3T3 NRU PT (OECD TG 432) and in house full thickness reconstructed skin model (H3D-PT) (INVITTOX n 118). This substance showed no degradation in UVA radiation, but presented high phototoxicity potential in 3T3 NRU PT (MPE = 0.909). On H3D-PT assay, the large molecular weight (> 500 Da) of fucoxanthin corroborate to its photosafety when tested at 0.5%. In this condition, the fucoxanthin presented no phototoxic response, while the control ketoprofen at 1% presented clear phototoxic response. Thus, fucoxanthin can be considered a promising candidate for a cosmetic ingredient.

References

Damiani, E., Baschong, W. and Greci, L. (2007). UV-filter combinations under UV-A exposure: Concomitant quantification of over-all spectral stability and molecular integrity. *J Photochem Photobiol B 87*, 95-104.

ECVAM DB-ALM: INVITTOX protocol. EPISKIN™ Skin Corrosivity Test. INVITTOX no. 118. https://ntp.niehs.nih.gov/iccvam/methods/dermal/epiderm/epiddocs/epidderm_invittox-508.pdf

OECD (2002). Guideline for the testing of chemicals: In vitro 3T3 NRU photoxicity test. Paris: OECD. Guideline Draft TG 432.

III-180

Towards immunocompetent organotypic human *in vitro* models: Example of skin melanoma

Jennifer Bourland[1], Caroline Gilbert[2], Julie Fradette[1] and Francois A. Auger[1]

[1]LOEX, Division of Regenerative Medicine, CHU de Quebec – Université Laval Research Center, Quebec, QC, Canada; [2]Department of microbiology, infectiology and immunology, CHU de Quebec – Université Laval Research Center, Quebec, Canada

jennifer.bourland.1@ulaval.ca

High attrition rate in drug development is partly due to failure to translate from preclinical to clinical phases. To increase the success rate, new human *in vitro* disease models are needed. Combining primary skin cells, melanoma microtissues and immune cells (PBMC), we developed a tissue-engineered skin model featuring blood and lymphatic microvascular networks characterized by CD31 and podoplanin immuno-stainings (Gibot et al, 2010, 2017). Tumors were localized at the dermoepidermal junction. Melanoma microtissues secreted significant amounts of VEGF-C while lymphatics secreted CCL21 and blood capillaries produced Ang-2, as determined by ELISA. Immune cells with distinct morphologies were positive for $CD14^+$, $HLA\text{-}DR^+$ or $CD8^+$. This model allows chronic testing and was treated for 12 days with PLX4720. It responded in a dose-dependent manner and showed significant differences in IC_{50} between cell lines. This unique model of melanoma mimics the microenvironment and key signaling involved in tumor progression.

References

Gibot, L., Galbraith, T., Huot, J. et al. (2010). A preexisting microvascular network benefits in vivo revascularization of a microvascularized tissue-engineered skin substitute. *Tissue Eng Part A 16*, 3199-3206.

Gibot, L., Galbraith, T., Bourland, J. et al. (2017). Tissue-engineered 3D human lymphatic microvascular network for in vitro studies of lymphangiogenesis. *Nat Protoc 12*, 1077-1088.

III-193

CON4EI: SkinEthic™ Human Corneal Epithelial Eye Irritation Test (SkinEthic™ HCE EIT) for hazard identification and labelling of eye irritating chemicals

An Van Rompay[1], Nathalie Alépée[2], Laurent Nardelli[2], Karen Hollanders[1], Virginie Leblanc[2], Przemyslaw Fochtman[3], Robert Guest[4], Helena Kandarova[5], Jamin Willoughby[6], Sandra Verstraelen[7] and Els Adriaens[8]

[1]VITO NV, Mol, Belgium; [2]L'Oréal Research & Innovation, Aulnay sous Bois, France; [3]Institute of Industrial Organic Chemistry Branch, Pszczyna, Poland; [4]Envigo, Cambridge, United Kingdom; [5]MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia; [6]Cyprotex US, LLC, Kalamazoo, MI, United States; [7]Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium; [8]Adriaens Consulting bvba, Aalter, Belgium

an.vanrompay@vito.be

Assessment of the acute eye irritation potential is part of the international regulatory requirements for testing of chemicals. The CEFIC-LRI-AIMT6-VITO CON4EI project was developed with the goal of assessing the reliability of eight *in vitro* test methods as well as establishing an optimal tiered-testing strategy. The goal of this study was to evaluate the performance of the SkinEthic™ HCE EITtest method in terms of the important *in vivo* drivers of classification. The chemicals were tested twice, the first run was performed by VITO and the second run was performed by L'Oréal. For the SkinEthic™ HCE EIT test method, 100% concordance in predictions (No Cat versus No prediction can be made) between the two participating laboratories was obtained. The accuracy of the SkinEthic™ HCE EIT was 97.5% with 100% sensitivity and 96.9% specificity. The SkinEthic™ HCE EIT confirms its excellent results of the validation studies.

This research is funded by CEFIC-LRI. Cosmetics Europe contributed in chemical selection.

III-231

Phase-1 of the validation study of Amino acid Derivative Reactivity Assay (ADRA): A novel *in chemico* alternative test method of skin sensitization

Atsushi Ono[1], Shinichi Watanabe[2], Tsunetsugu Sugawara[2], Koji Wakabayashi[3], Yu Tahara[3], Nobuyuki Horie[4], Keiichi Fujimoto[4], Kei Kusakari[5], Yoshihiko Kurokawa[5], Takashi Sozu[6], Takuto Nakayama[6], Takeru Kusao[6], Tsuyoshi Kawakami[7], Kohichi Kojima[8], Hajime Kojima[9], Jon Richmond[10], Nicole Kleinstreuer[11], Kim Bae-Hwa[12], Yusuke Yamamoto[13], Masaharu Fujita[13] and Toshihiko Kasahara[13]

[1]Graduate school of Medicine, Dentistry and Pharmaceutical sciences, Okayama University, Okayama, Japan; [2]Human & Environmental Safety Evaluation Center, Lion Corporation, Kanagawa, Japan; [3]Chemical Safety Department, Mitsui Chemicals, Inc., Tokyo, Japan; [4]Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Tokyo, Japan; [5]Biological Research Laboratories, Nissan Chemical Industries, Ltd, Tokyo, Japan; [6]Faculty of Engineering, Tokyo University of Science, Tokyo, Japan; [7]Division of Environmental Chemistry, National Institute of Health Sciences, Tokyo, Japan; [8]Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan; [9]Division of Risk Assessment, National Institute of Health Sciences, Tokyo, Japan; [10]UK Home Office, Dundee, United Kingdom; [11]NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), Durham, NC, United States; [12]College of Natural Sciences Keimyung University, Daegu, South Korea; [13]Safety Evaluation Center, Fujifilm Corporation, Tokyo, Japan
atsushiono3@okayama-u.ac.jp

ADRA (Amino acid Derivative Reactivity Assay) has been developed as an *in chemico* test method for detecting chemicals with skin sensitization potential, and represents a significant advancement on the DPRA (Direct Peptide Reactivity Assay) adopted in OECD TG-442C in 2015.

ADRA uses the synthetic chemicals NAC (N-2(1-napthyl) acetyl)-L-cysteine) and NAL (α-N-(2-(1-naphthyl)acetyl)-L-lysine) to overcome problems with precipitation and co-elution inherent in the DPRA test methods. ADRA is currently undergoing a phased multi-site validation study.

The results of the Phase-1 of ADRA validation study are reported here. Using an optimized SOP, four laboratories blindly tested three replicate sets of 10 representative chemicals, with each replicate coded separately. The WLR (Within-Laboratory Reproducibility) achieved by each laboratory was greater than 90%, surpassing the target value of 80% required by OECD for test methods sharing the DPRA essential test method requirements.

Phase-2 of the validation study, with four laboratories testing an additional 30 chemicals to determine the BLR (Between-Laboratory Reproducibility) and Predictive Capacity, is underway.

III-233

Combined *in vitro* method strategy for assessment of hair growth material

JiaTing Xu[1], Shujun Cheng[2], Yao Qin[1] and Mengmeng Geng[1]

[1]Research and Development Department, Guangzhou CHN-ALT Biotechnology Co. Ltd, Guangzhou, People's Republic of China; [2]Guangdong Inspection and Quarantine Technology Center, Pearl River New Town, Guangzhou, People's Republic of China
cristin1225@ccare.net.cn

As an independent third-party testing company which devote to non-animal alternative research, we apply *in vitro* technology to the safety and efficacy assessment of traditional plant materials with germinal efficacy. According to the discipline of epicranium hair follicle stem cell growth mechanism, different factors cause hair growth impede and induce 5α-II reductase activity, cell activity and protein level (e.g. phospho-GSK3β, β-catenin, Cyclin E, CDK2 and $p27^{kip1}$) changed. Combined valided test methods to establish a hierarchical and integrated strategy which for hair growth material and products efficacy screening, specifically including hair-fiber elongation of rat vibrissa follicle, rat immortalized vibrissa dermal papilla cells (DPCs) test, NIH3T3 fibroblasts test and human eyelash test. Under the *in vitro* screening system, several plant extracts have been screened with germinal efficacy at appropriate concentrations, respectively, and indicated that can take the next step clinical assessment.

References

Goetze, O., Fox, M., Kwiatek, M. A. et al. (2009). Effects of postgastric 13C-acetate processing on measurement of gastric emptying: A systematic investigation in health. *Neurogastroenterol Motil 21*, 1047-1085.

Kim, J. Y., Park, J. Y., Kang, H. J. et al. (2012). Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: A randomized, double-blind, placebo-controlled trial. *Nutr J 11*, 47.

Lee, S. H., Kang, S. M., Ko, S. C. et al. (2012). Octaphlorethol A, a novel phenolic compound isolated from a brown alga, Ishige foliacea, increases glucose transporter 4-mediated glucose uptake in skeletal muscle cells. *Biochem Biophys Res Commun 420*, 576-581.

Ouji, Y., Yoshikawa, M., Shiroi, A. and Ishizaka, S. (2006). Promotion of hair follicle development and trichogenesis by Wnt-10b in cultured embryonic skin and in reconstituted skin. *Biochem Biophys Res Commun 345*, 581-587.

Wangefjord, S., Brändstedt, J., Ericson Lindquist, K. et al. (2013). Associations of beta-catenin alterations and MSI screening status with expression of key cell cycle regulating proteins and survival from colorectal cancer. *Diagn Pathol 8*, 10.

III-253

Use of 3 animal product free methods as part of a novel integrated approach to skin sensitisation testing

Alexander Edwards, Nathalie Belot, Lottie Roscoe, Christopher Longmore, Bushra Sim and Carol Treasure
XCellR8, Daresbury, United Kingdom
alex.edwards@x-cellr8.com

An integrated approach to *in chemico* and *in vitro* skin sensitisation using three methods; DPRA, KeratinoSens and h-CLAT, has recently been approved by the OECD. Here, we present novel data from a representative case study using animal free adaptations of the 2 *in vitro* skin sensitisation methods alongside data from the *in chemico* DPRA method. Animal product free adaptations to h-CLAT and KeratinoSens include the use of Human Serum, Human Serum Albumin, custom anti-CD54 and 86 antibodies produced using phage display and Trypzean from plant sources to replace the use of animal derived components. All 3 methods have been validated in-house and approved for use in REACH submissions. We are seeking inclusion of the *in vitro* adaptations into OECD TG 442D and 442E. The test item from the case study is the first to be used in this completely animal free workflow and was successfully classified using the 3 methods with a 2/3 approach being taken to decide upon the outcome of the testing.

III-267

Adaptation of the KeratinoSens Skin Sensitisation Test to animal-product-free conditions

Carol Treasure, Bushra Sim, Lottie Roscoe, Christopher Longmore and Nathalie Belot
XCellR8 Ltd, Daresbury, United Kingdom
carol.treasure@x-cellr8.com

Currently, many *in vitro* methods use animal-derived components in the cell culture systems. Many stakeholders in the cosmetics industry have both scientific and ethical concerns relating to this issue and have stated a strong preference for fully human *in vitro* test systems. We have adapted the KeratinoSens method to animal product-free cell culture conditions, and carried out an in-house validation with 21 reference substances, including those listed in the Performance Standards associated with OECD TG442d. The modified method was shown to be totally equivalent to the Validated Reference Method (VRM), with comparable values for accuracy (85.7%), sensitivity (84.6%) and specificity (87.5%), and all acceptance criteria being met. In Europe, data generated by the adapted method may be used in REACH submissions, and we are now seeking approval to list the adaptation in OECD Test Guideline 442d, enabling formal compliance with a range of global regulations.

Reference

Natsch, A., Ryan, C. A., Foertsch, L. et al. (2013). A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. *J Appl Toxicol 33*, 1337-1352.

III-268

Adaptation of human cell based safety tests to animal product free conditions

Carol Treasure, Bushra Sim, Christopher Longmore, Lottie Roscoe and Nathalie Belot
XCellR8 Ltd, Daresbury, United Kingdom
carol.treasure@x-cellr8.com

Much progress has been made in terms of the regulatory acceptance of human cell based methods for the assessment of human safety. However, most of these methods still use animal-derived components, such as serum, tissue extracts and antibodies, for which both scientific and ethical drawbacks have been well-documented. Such methods cannot be considered truly animal-free. XCellR8 has adapted 3 key human cell based safety tests to animal-free conditions: the regulatory KeratinoSens and h-CLAT tests for skin sensitisation, and the non-regulatory BlueScreen test for genotoxicity. In addition, we have developed a new pre-screen for acute toxicity using human cells in animal-free culture. Our in-house validation of these methods has shown equivalence and improvements to the fully validated published methods. Here we present an overview of this work so far and the steps taken to gain regulatory approval, demonstrating that truly animal-free regulatory safety testing is now achievable.

References

Barker-Treasure, C., Coll, K., Belot, N. et al. (2015). Non-animal replacements for acute toxicity testing. *Altern Lab Anim 43*, 199-203.
Belot, N., Sim, B., Longmore, C. L. et al. (2017). Adaptation of the KeratinoSens™ skin sensitisation test to animal-product-free cell culture. *ALTEX*, Epub ahead of print. doi:10.14573/altex.1701311

III-306

3D Skin Comet assay: Genotoxicity assessment addressing the dermal route of exposure

Sebastian Hoffmann[1], Kerstin Reisinger[2], Joep Brinkmann[3], Tom Down[4], Anja Fischer[2], Andrea Haase[3], Frank Henkler[3], Manfred Liebsch[3], Andreas Luch[3], Claudia Petrick[2], Ralph Pirow[3], Astrid Reus[5], Andre Said[3,6], Monika Schäfer-Korting[6], Markus Schulz[7] and Stefan Pfuhler[4]

[1]seh consulting + services, Paderborn, Germany; [2]Henkel AG & Co KGaA, Düsseldorf, Germany; [3]Department of Chemical and Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany; [4]Procter & Gamble, Mason, OH, United States; [5]Triskelion B.V., Zeist, The Netherlands; [6]Institute for Pharmacy, Freie Universität Berlin, Berlin, Germany; [7]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany
sebastian.hoffmann@seh-cs.com

The 3D Skin Comet assay was developed as follow-up tool for positive results from standard *in vitro* test batteries. It addresses two aspects underrepresented in current *in vitro* genotoxicity testing: *in vivo*-like route of exposure and metabolism. To mimic the dermal exposure route full-thickness skin tissues were combined with a classical readout-parameter, the migration of DNA fragments in the electric field. The fragments represent DNA damage that may lead to clastogenic as well as to mutagenic lesions.

We here report on a validation study in which 30 compounds were tested blinded in five laboratories. Data analysis revealed a specificity of 87% and a sensitivity of 73% which is comparable to the predictivity obtained *in vivo*.

According to our findings, the 3D Skin Comet assay can be used as a direct replacement of animal studies when following-up on positive results from the standard test battery.

The work was funded by the German Ministry for Research and Education and Cosmetics Europe.

III-322

Integrated hazard identification of chemical sensitizers using *in vitro* and *in silico* readouts – A comparative evaluation of predictive performance

Donna Macmillan[1], Henrik Johansson[2], Olivia Larne[2] and Malin Lindstedt[3]

[1]Lhasa Limited, Leeds, United Kingdom; [2]SenzaGen, Lund, Sweden; [3]Lund University, Lund, Sweden
donna.macmillan@lhasalimited.org

The assessment of skin sensitisation currently relies on animal experimentation. However, due to the increased drive to reduce, refine and replace animal assays, many non-animal methods are now available. The GARD assay (Genomic Allergen Rapid Detection) is a novel, dendritic cell-based assay which identifies skin sensitisers from 200 genomic biomarkers currently undergoing validation by the OECD (Johansson et al., 2014). The GARD assay has demonstrated high predictive capacity to distinguish between sensitisers and non-sensitisers (Johansson et al., 2017), but combinations of more than one non-animal method can behave in a complementary fashion to improve predictivity (Macmillan et al., 2016).

GARD was evaluated (alongside the OECD-validated assays DPRA, KeratinoSens and h-CLAT) against an in-house dataset of over 400 chemicals with accuracies between 72-87% for each assay. The individual assay results were then combined with an *in silico* toxicity prediction provided by Derek Nexus, which led to a significant increase in the accuracy compared to using the assays alone (77-93%). When using only Derek Nexus and GARD, the positive predictivity and negative predictivity were 91% and 92%, respectively.

References

Johansson, H., Rydnert, F., Kuehnl, J. et al. (2014). GARD in-house validation – A proof of concept. *Toxicol Sci 39*, 362-370.

Johansson, H., Gradin, R., Forreryd, A. et al. (2017). Evaluation of the GARD assay in a blind Cosmetics Europe study. *ALTEX*, in press. doi:10.14573/altex.1701121

Macmillan, D. S., Canipa, S. J. and Chilton, M. L. (2016). Predicting skin sensitisation using a decision tree integrated testing strategy with an in silico model and in chemico/in vitro assays. *Regul Toxicol Pharmacol 76*, 30-38.

III-346

Historical performance of weak sensitizers in different animal models

Bennett Varsho, Micheal Carathers, Debra Hall and George DeGeorge

MB Research Labs, Spinnerstown, United States
bvarsho@mbresearch.com

In the mid-1990s, regulators moved from allowing the use of strong positive control agents in animal studies for dermal sensitization to requiring the use of weak to moderate sensitizers. This shift arose from the desire to prove that the performing laboratories could identify weak to moderate sensitizers under their testing conditions, reducing concerns regarding false negatives. Twenty years later, replacement agents (including MBT and HCA) are in widespread use and demonstrate the test methods' capacity to identify weak to moderate sensitizers. In our laboratory, the Guinea Pig Maximization Test (GPMT) returns 30-45% positive responders (PR) with MBT, while the Buehler Method returns 20-65% PR with HCA. In the LLNA, 25% HCA returns 40-100% PR using both the flow cytometric method and the more recent BrdU-ELISA method. Factors potentially affecting positive response rates, including induction and/or challenge concentrations, vehicle effects, are compared and reported in detail.

III-406

COCAT – Advanced *in vitro* assessment of skin sensitization potency of chemicals using THP-1 cells in coculture with HaCaT keratinocytes

Jennifer Hennen and Brunhilde Blömeke

Department of Environmental Toxicology, Trier University, Trier, Germany

jennifer.hennen@uni-trier.de

Identification of skin sensitization hazard and potency assessment is crucial for quantitative risk assessment of chemicals. We evaluated the capacity of COCAT (THP-1 cells in coculture with HaCaT keratinocytes) for the simultaneous prediction of sensitization hazard and potency. Analyzing the upregulation of CD86 and CD54 on THP-1 cells after exposure to 21 sensitizers (8 haptens, 8 prohaptens, 5 prehaptens) and 12 non-sensitizers found 97% accuracy for hazard prediction. The minimum concentration inducing a positive response in COCAT predicted GHS potency sub-categories with 85% accuracy and correlated very good with continuous data on *in vivo* skin sensitization potency (LLNA). In-depth analysis of COCAT results for structurally related sensitizers revealed distinct responses reflecting *in vivo* differences. These promising data demonstrate that COCAT, integrating keratinocyte responses with the activation of THP-1 cells, allows the assessment of sensitization hazard and potency.

III-415

Expert review of (Q)SAR predictions for skin sensitization potency

Thomas Lewandowski[1] and Joel Cohen[2]

[1]Gradient, Seattle, WA, United States; [2]Gradient, Cambridge, MA, United States

tlewandowski@gradientcorp.com

Skin sensitization potency is typically determined based on data collected from the Local Lymph Node Assay, human patch testing, or guinea pig studies. Such data are not available for many existing chemicals that may require evaluation within restrictive budgetary and time constraints. For these situations, alternative methods such as predictive *in silico* (Q)SAR programs may be considered. However, (Q)SAR estimates often require an additional level of expert scrutiny. We evaluated the impact of applying expert review to the predicted skin sensitization thresholds (EC3 values) from the QSAR program Derek Nexus for a small set of well-known sensitizers. We then compared the predicted EC3 values against potency classifications determined based on readily available guinea pig or human data. We found SAR estimates were appropriate in 35% of cases; in 35% of cases actual data provided a more health protective threshold estimate and in 29% of cases, SAR estimates were more conservative but judged less reliable. Our results highlight the importance of expert review in the interpretation and application of (Q)SAR estimates for skin sensitization potency.

III-424

U937 cell line activation test: U-SENS, an OECD adopted *in vitro* skin sensitisation assay addressing the activation of dendritic cells

Nathalie Alépée, Cecile Piroird, Laurent Nardelli and José Cotóvio

L'Oréal, Aulnay sous Bois, France

nalepee@rd.loreal.com

The current knowledge of the mechanisms associated with skin sensitisation has been summarised as an Adverse Outcome Pathway (AOP). U937cell line activation Test (U-SENS™) addresses this 3rd Key Event on this AOP by quantifying the change in the expression of a cell surface marker associated with the process of activation of monocytes and DC (CD86) in the human histiocytic lymphoma cell line, following exposure to sensitisers. Results generated in validation studies indicate that, compared with LLNA results, the accuracy in distinguishing skin sensitisers (UN GHS Cat.1) from non-sensitisers is 86% (N = 166) with a sensitivity of 91% (118/129) and a specificity of 65% (24/37). Considering all available evidence and input from regulators and stakeholders, the U-SENS™ was recommended by EURL ECVAM and adopted in an OECD Test Guideline to be used as part of an IATA to support the discrimination between sensitisers and non-sensitisers for the purpose of hazard classification and labelling.

III-441

SkinEthic™ RHE: A relevant tool for assessing topical phototoxic chemicals *in vitro*

Christelle Videau[1], Solène Dreyfuss[1], Valérie Tagliati[1], Coralie Bernard[1], Thibaud Weiler[2], Julien Ortega[2], Damien Lelievre[1], Carine Tornier[1] and Nathalie Seyler[1]

[1]Episkin SA, Lyon, France; [2]Galderma, Biot, France

cvideau@episkin.com

The aim of this study was to investigate the ability of SkinEthic™ RHE (Reconstructed Human Epidermis) to identify the phototoxic potential of chemicals.

Based on previous works performed by F-X Bernard et al. (2000), the protocol was optimized to test all types of chemical.

Following topical or systemic application for 18 hours, tissues were exposed to non-cytotoxic dose (pre-test) of UVA (6 J/cm^2). After rinsing and post-incubation steps, the cell viability was measured (1 mg/ml MTT).

Results obtained on 8 chemicals (from 3T3 NRU PT list) showed that SkinEthic™ RHE was able to fully discriminate between non-phototoxic and phototoxic compounds.

According to these promising results and in order to prove the protocol robustness, this latter was transferred successfully to another external laboratory.

Results allow us to conclude that the SkinEthic™ RHE model is a relevant and discriminant tool to assess *in vitro* the phototoxic potential of chemicals and to overcome 3T3-NRU PT test limitations.

III-452

An evaluation of selected (Q)SARs/expert systems for the prediction of skin sensitization potential

Jeremy Fitzpatrick and Grace Patlewicz

National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park (RTP), NC, United States
fitzpatrick.jeremy@epa.gov

One of the alternative approaches to assessing skin sensitization hazard is through the use of (Q)SARs/expert systems. Here we evaluate the predictive performance of two expert systems (TIMES-SS and VEGA) and two SAR rulebases (OASIS protein binding alerts and Toxtree's reactivity domains). To evaluate them, a dataset of 473 substances tested in the local lymph node assay was compiled, comprising 295 sensitizers and 178 non-sensitizers. The overall predictivity of TIMES-SS and VEGA were 63% and 64%, respectively. Both programs were better at predicting sensitizers than non-sensitizers. The SAR rulebases from Toxtree and OASIS showed similar results for overall predictivity with 69% and 64% accuracy, respectively. The OASIS alerts were unique in that they were better at predicting non-sensitizers than sensitizers. Currently there appears to be no front runner for the prediction of skin sensitization.

This abstract may not reflect U.S. EPA policy.

III-457

Assessing the allergenic potential of proteins from their sequence and 3D structure using novel computational approaches

G. Frank Gerberick[1], Vithiagaran Gunalan[2], Vachiranee Limviphuvadh[2], Cindy Ryan[1], Petra Kern[3] and Sebastian Maurer-Stroh[2]

[1]Procter & Gamble Company, Mason, OH, United States; [2]Bioinformatics Institute, A*STAR, Singapore; [3]Procter & Gamble Company, Strombeek-Bever, Belgium
gerberick.gf@pg.com

Currently, there are no validated or widely accepted *in vivo* or *in vitro* models available for assessing individual proteins or mixtures of proteins from a natural/botanical sample for their allergenic potential. Previous FAO/WHO guidelines based on single hexamer peptide hits and sequence identity thresholds produce a large number of false positives reaching up to 90% of all HUMAN proteins. We propose an entropy-adjusted hexamer hit approach for a 6-fold reduction of false positives as well as switching from linear sequence window similarity to B-cell epitope-like 3D surface similarity with predicted structures for 76% of all known allergens. Using a benchmark set of known allergens and likely non-allergens sharing the same structural fold, we show that the 3D epitope similarity method increased accuracy of classification by 2-fold. Using this novel computational approach has potential to reduce our reliance on animal data for conducting robust respiratory allergy risk assessments.

III-470

DC SkinSens – An online integrated testing strategy for quantitative skin sensitization potency assessment using Bayesian networks and accounting for bioavailability

Ahmed Abdelaziz Sayed, Maja Brajnik, Daniel Bachler, Thomas Exner and Barry Hardy

Douglas Connect, Basel, Switzerland
ahmed@douglasconnect.com

Skin sensitization is a public health problem that is attributed with high direct and indirect costs. Multiple *in vitro* tests are available for assessing chemical hazard of skin sensitization to replace and reduce the *in vivo* animal test (Local Lymph Node Assay – LLNA). Strategies for integrating such tests, together with *in silico* calculations of chemical bioavailability, have been proposed in literature. The integrated testing strategy for skin sensitization (ITS-3) is one such approach. It utilizes Bayesian networks for predicting skin sensitization hazard and guides the testing process. In this work, we reproduce the results from the ITS-3 article (Jaworska et al., 2015) and develop a web-application that guides the user through the application of the ITS-3 strategy to assess chemical potency of skin sensitization potency (LLNA pEC3). Open and free tools were favoured during the course of this work to ensure reproducibility and encourage adoption by regulators and wider communities. The network diagram was reproduced from the description reported (Jaworska et al., 2015). The *in silico* descriptors for bioavailability were replaced with custom-models with high correlation to the reported values, the mutual information calculations were reproduced in R and the post-prediction correction for Michael acceptors was implemented using SMARTS patterns. The network also reports on the predictions' confidence using Bayesian factors and therefore can reduce animal testing to compounds with higher priority where evidence from alternative approaches is insufficient to produce confident hazard assessment. The overall network accuracy for 4-category classification (non-, weak, moderate or strong sensitizers) was 80% while accuracy of the most confident predictions was 98%. The web application is freely accessible online on https://its.douglasconnect.com

Reference

Jaworska, J. S., Natsch, A., Ryan, C. et al. (2015). Bayesian Integrated Testing Strategy (ITS) for skin sensitization potency assessment: A decision support system for quantitative weight of evidence and adaptive testing strategy. *Arch Toxicol 89*, 2355-2383.

III-481

Effects of nicotine on melanocytes

Jin Sil Lee[1], Eugenia Jin Lee[2] and Lim Kyung-Min[1]

[1]College of Pharmacy, Ewha Womans University, Seoul, South Korea; [2]Seoul International School, Seoul, South Korea
wlstlf113@nate.com

Cigarette smoking has many undesirable effects on the human body including the skin. Nicotine, the main toxic constituent of the solid phase, is absorbed through the oral cavity, lung, bladder, gastrointestinal track, and skin. Nicotine darkens the skin tone and relationship between melanin synthesis and nicotine has been suggested. However, the increased production of melanin by nicotine or its underlying mechanism has not been fully established yet. Here, we and investigated the morphological change in a melanoma cell line and human artificial tissue, Melanoderm™ induced by nicotine exposure. And we also studied the mechanism of melanin formation associated with nicotine to provide a novel insight into the cigarette-smoking associated skin hyperpigmentation.

The present study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant No. HN15C0102)

III-483

Determination of contact sensitization potential of chemicals using *in vitro* reconstructed normal human epidermal model EpiDerm: Impact of the modality of application

Silvia Letasiova[1], Emanuela Corsini[2], Valentina Galbiati[2], Helena Kandarova[1], Dagmar Lehmeier[3] and Helge Gehrke[3]

[1]MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia; [2]Laboratory of Toxicology, Department of Pharmacological and Biomolecular Sciences, Universita degli Studi di Milano, Milan, Italy; [3]Eurofins Biopharma Product Testing Munich GmbH, Germany
sletasiova@mattek.com

Assessment of skin sensitization potential has traditionally been conducted in animal models, such as the Mouse Local Lymph Node Assay (LLNA) and the Guinea Pig Maximisation Test (GPMT). However, a growing focus and consensus for minimizing animal use have stimulated the development of *in vitro* methods to assess skin sensitization. Interleukin-18 (IL-18) release in reconstructed human epidermal models has been identified as a potentially useful endpoint for the identification and classification of skin sensitizing chemicals, including chemicals of low water solubility or stability (1).

The purpose of this study was to investigate the impact of the modality of chemical exposure on the predictive capacity of the assay. EpiDerm tissue viability assessed by MTT assay and IL-18 release assessed by ELISA were evaluated after 24 h topical exposure to test chemicals either impregnated in 8 mm diameter paper filters or directly applied to the surface of EpiDerm. Acetone: olive oil (4:1) was used as vehicle in all cases. A total of five chemicals from 3 different sources were tested. The testing set included 3 sensitizers, namely 2,4-dinitrochlorobenzene, cinnamaldehyde and isoeugenol/eugenol, and 2 non-sensitizers, lactic acid and salicylic acid. Four independent dose-response experiments were conducted in 3 laboratories, resulting in correct prediction of the sensitizing potency of test chemicals.

The assessment of IL-18 release using *in vitro* reconstructed normal human epidermal model EpiDerm appears to be a promising tool for *in vitro* determination of contact sensitization potential.

Reference

Gibbs, S., Corsini, E., Spiekstra, S. W. et al. (2013). An epidermal equivalent assay for identification and ranking potency of contact sensitizers. *Toxicol Appl Pharmacol 272*, 529-534.

III-489

Cosmetics Europe long range science strategy for non-animal-based safety assessments

Bertrand Desprez[1], Martina Klaric[1], Rob Taalman[1], Andreas Schepky[2] and Bas Blaauboer[3]

[1]Cosmetics Europe, Brussels, Belgium; [2]Beiersdorf AG, Hamburg, Germany; [3]University of Utrecht, Institute for Risk Assessment Sciences, Utrecht, The Netherlands
bdesprez@cosmeticseurope.eu

The Cosmetics Europe Long Range Science Strategy (LRSS) aims at determining safety of cosmetic ingredients without the use of animals. It will include exposure for relevant use scenarios; local effects; biokinetics; systemic effects where relevant.

To provide proof of concept, case studies will be performed using a tiered structure, tier 0 focusing on data collection on structure, exposure, known data for the compound and for similar chemicals, allowing TTC or a read-across approach in a safety assessment. If this is insufficient tier 1 focuses on scientifically based choices of *in silico* and/or *in vitro* work, based on hypotheses for understanding of modes of action together with information on biokinetics.

In tier 2 experimental work will lead to the determination of a point of departure for a quantitative *in vitro-in vivo* extrapolation as the basis for a safety assessment.

The aim is to show that this will result in safety assessments for use in regulatory frameworks.

III-552

Assessment of the phototoxicity of three different TiO_2 nano-forms using reconstructed human tissue model EpiDerm

Silvia Letasiova[1], Alzbeta Liskova[1], Tatiana Milasova[1], Sona Jantova[2], Vlasta Brezova[3] and Helena Kandarova[1]

[1]MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia; [2]Department of Biochemistry and Microbiology, FCHPT, Institute of Biochemistry, Nutrition and Health Protection, Bratislava, Slovakia; [3]Department of Physical Chemistry, FCHPT, Institute of Physical Chemistry and Chemical Physics, Bratislava, Slovakia
sletasiova@mattek.com

Absorption of the solar light by photo-sensitive substances and consequent formation of reactive oxygen species and other photo-products may lead to the cellular damage as well as to responses of the immune system. Determination of phototoxicity of substances absorbing UV and visible spectra of the solar light (VIS) belongs therefore to the basic toxicology tests.

One of the methods used for the determination of phototoxicity is a test based on the use of *in vitro* reconstructed human skin tissue model EpiDerm. This test (EpiDerm H3D-PT) was developed and pre-validated by organization ZEBET already in 1997. The main objective of this work was to determine the phototoxic potential of the selected reference substances and three different types of TiO_2 nanoparticles using the EpiDerm H3D-PT.

At first, we evaluated and standardized the measurement conditions of the sunlight simulator SOL-500 and verified the sensitivity of the EpiDerm tissues towards UV/VIS light. Next, we evaluated correct prediction of the EpiDerm H3DPT using six reference substances, of which four were known phototoxins and two compounds were UV-absorbing, but without phototoxic potential. Finally, we used this method to predict the phototoxicity of three different types of titanium dioxide (P25 AEROXID, Eusolex T-2000, TIG-115).

For the reference compounds, we obtained the same or better results as published by Liebsch et al. (1997). Phototoxicity of TiO_2 has not been demonstrated in any of the three samples tested. We conclude that the EpiDerm H3D-PT is a reliable test for the detection of phototoxicity and prediction of the phototoxic potential.

III-572

Validation of the 3D human reconstructed skin micronucleus assay (RSMN) using the EpiDerm™ tissue

Stefan Pfuhler[1], Marilyn Aardema[2], Shambu Roy[3], Rohan Kulkarni[3], Greg Munn[4], Nathan Wilt[4], Emilia Costin[4], Rodger Curren[4], Brenda Barnett[5], Sebastian Hoffmann[6] and Bertrand Desprez[7]

[1]Procter & Gamble, Mason, OH, United States; [2]Marilyn Aardema Consulting, Fairfield, OH, United States; [3]BioReliance, Rockville, United States; [4]IIVS, Gaithersburg, MD, United States; [5]Advanced Testing Laboratories, Cincinnati, OH, United States; [6]seh consulting + services, Paderborn, Germany; [7]Cosmetics Europe, Brussels, Belgium
pfuhler.s@pg.com

Skin tissue-based test systems provide a more realistic model for topically applied chemicals, such as cosmetics. The Reconstructed Skin Micronucleus assay (RSMN) using the EpiDerm™ model has shown good transferability and inter- and intra-laboratory reproducibility in validation studies to date. There was an excellent overall specificity (about 90%) based on 38 coded chemicals. The sensitivity observed with the standard 48 h treatment protocol was lower than hoped (65%) which led to assay procedure modifications of extending the treatment from 48 h to 72 h and more optimal dose selection. Bridging studies including a 72 h treatment were performed to evaluate the modified protocol and showed that the sensitivity of the assay increased to 80%. Importantly, the modified protocol did not compromise the specificity of the RSMN. These validation studies support the use of the RSMN as a direct replacement of *in vivo* assays.

III-606

Hyperosmolal vaginal lubricants markedly increase epithelial damage in a 3D vaginal epithelium model (EpiVaginal™)

Seyoum Ayehunie[1], Ying-Ying Wang[2], Stephanie Bogojevic[1], Timothy Landry[1], Mitchell Klausener[1], Richard Cone[2] and Patrick Hayden[1]

[1]MatTek Corporation, Ashland, MA, United States; [2]Johns Hopkins University, Baltimore, MD, United States
sayehunie@mattek.com

Widely used vaginal lubricants are 4-30x the osmolality of healthy vaginal fluid. Hyperosmolal formulations cause toxicity to human colorectal epithelia *in vivo*, increase vaginal transmission of genital herpes in a mouse/HSV model, cause toxicity to vaginal epithelia explants or cultured vaginal epithelial cells and increase susceptibility to pathogenic organisms such as HIV. Using an *in vitro* 3D human vaginal epithelium tissue model (EpiVaginal™), we show that hyperosmolal vaginal lubricants with osmolality > 4 times that of vaginal fluid (> 1500 mOsm/Kg) cause disruption of epithelial barrier and structural damage. 4 out of 4 such lubricants caused histological tissue disruption, and compromised barrier integrity. No epithelial damage or reduction viability was noted for lubricants (N = 3) with osmolality of < 370 mOsm/Kg. The results show the utility of the EpiVaginal tissue model for lubricant screening and confirm the extensive reports of safety concerns of hyperosmolal lubricants.

III-651

Analysis of co-exposure effects *in vitro* – The irritant sodium dodecyl sulfate increases the upregulation of costimulatory molecules by the sensitizer 2,4-dinitrochlorobenzene

Jennifer Hennen[1], Jutta Lichter[1], Zineb El Youssoufi[1], Carsten Goebel[2] and Brunhilde Blömeke[1]

[1]Department of Environmental Toxicology, Trier University, Trier, Germany; [2]Toxicology Department, Coty Professional Beauty, Darmstadt, Germany

jennifer.hennen@uni-trier.de

It is commonly accepted that skin irritation enhances skin sensitization. *In vivo*, pre- or co-exposure to the irritant sodium dodecyl sulfate (SDS) augmented the immune response to contact sensitizers such as 2,4-dinitrochlorobenzene (DNCB).

To study the impact of co-exposure to skin irritants and allergens on dendritic cell (DC) activation, we exposed THP-1 cells to SDS and DNCB and measured the upregulation of costimulatory molecules CD86 and CD54. We found that co-exposure to SDS promoted THP-1 activation by low concentrations of DNCB. Furthermore, pre-exposure of HaCaT keratinocytes to SDS enhanced the THP-1 response to DNCB, indicating that keratinocytes exposed to SDS can enhance sensitizer-induced DC activation.

In sum, our data indicate that the introduced *in vitro* models are able to mimic augmenting effects of SDS on skin sensitization observed *in vivo*. Therefore, we consider them as promising tools to further study how irritation affects the immune response to skin sensitizers.

III-655

Assessment of skin sensitization potential of UV filters in a skin model with T cells exposure

Carolina Benevenuto[1], Leonie Wallmeyer[2], Sarah Hedtrich[2] and Lorena Gaspar[1]

[1]Pharmaceutical Sciences, School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo, Ribeirão Preto, Brazil; [2]Pharmacology, Institute for Pharmacy, Freie Universität Berlin, Berlin, Germany

carolgb@fcfrp.usp.br

Ultraviolet (UV) filters are considered emergent cosmetic allergens (Nash, 2006; Bryden et al., 2006). *In vitro* assessment of skin sensitization potential is a challenge and there is no consensus about a strategy to completely replace animal testing, especially for low solubility chemicals. We assessed the skin sensitization potential of UV filters using cytokine release in skin models with exposure to $CD4^+$ T lymphocytes (Wallmeyer et al., 2017). UV filters were topically applied and the release of IL-6, IL-8 and IL-18, as well as the cell viability were measured. UV filters were not considered skin sensitizers. The most promising approach was the assessment of IL-18 release, since it allowed distinguishing the sensitizer and the irritant controls. *In vitro* prediction of skin sensitization by UV filters remains challenging, although the approaches reported here might represent a new method to predict the sensitizing potential of compounds.

References

Bryden, A. M. et al. (2006). Photopatch testing of 1155 patients: Results of the U.K. multicentre photopatch study group. *Br J Dermatol 155*, 737-747. doi:10.1111/j.1365-2133.2006.07458.x

Nash, J. F. (2006). Human safety and efficacy of ultraviolet filters and sunscreen products. *Dermatol Clin 24*, 35-51. doi:10.1016/j.det.2005.09.006

Wallmeyer, L., Dietert, K., Sochorová, M. et al. (2017). TSLP is a direct trigger for T cell migration in filaggrin-deficient skin equivalents. *Sci Rep 7*, 774. doi:10.1038/s41598-017-00670-2

III-660

Assessment of photogenotoxic potential of the UVA filter avobenzone using human keratinocytes cells (HaCaT)

Camila Kawakami, Camila Mini, Danielle Palma de Oliveira and Lorena Rigo Gaspar

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil

milakawakami@hotmail.com

The photogenotoxicity tests are recommended in safety assessment when a substance absorbs UV radiation, presents photoreactivity and distributes in light-exposed tissues; thus, UV filters fulfill these requirements (Lynch et al., 2011). Avobenzone (AVO) is the most widely used UVA filter, however its photoinstability can result in loss of efficacy and generation of photoproducts with unknown toxicological properties (Shaath, 2010). Thus, the aim of this study was to evaluate the photogenotoxic potential of AVO using the comet assay on HaCaT (Tice et al., 2000). For this purpose, cells were treated for 4 h with different concentrations of AVO (2.5-100 µg/mL) and were subjected or not to 4 J/cm^2 of UVA radiation and then the comet assay was performed. The results demonstrated that avobenzone did not induce to DNA breaks, even at the highest concentration of 100 µg/mL. However, this test might be further supported by additional endpoints to better understand the behavior of this UV filter.

References

Lynch, A. M., Guzzie, P. J., Bauer, D. et al. (2011). Considerations on photochemical genotoxicity. II: Report of the 2009 International Workshop on Genotoxicity Testing Working Group. *Mutat Res Genet Tox 723*, 91-100.

Shaath, N. A. (2010). Ultraviolet filters. Photochem *Photobiol Sci 9*, 464-469.

Tice, R. R., Agurell, E. and Anderson, D. (2000). Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen 35*, 206-221.

III-707

Improvement of predictive ability to distinguish skin irritant using reconstructed human epidermis model, SoluDerm™, by changing washing method

Jung Eun Kim[1], Seol Yeong Kim[1], Hye Soo Kim[1], Su Hyon Lee[1], Jin Sik Kim[2] and Eun Joo Kim[2]

[1]Biosolution Inc., Seoul, South Korea; [2]Korea Conformity Laboratories, Seoul, South Korea
rms@biosolutions.co.kr

Regarding animal welfare which became a social issue with developed countries, various researches substituting animal experiment are actively being proceeded. In this study, we aim to register at OECD TG 439 through the evaluation of experimental skin model SoluDerm™ (Biosolution Inc., Korea) having the structure and function like human skin. We tried to meet the OECD TG 439 criteria by solving the problems presented by improving the prediction ability by performing the optimized test method. After test material was applied for 45 minutes, using a squeeze bottle, was carried out which was compared with the conventional washing, with a pipette. The cell viability was measured by MTT assay and the cell viability was determined to be non-stimulated based on 50% or less. For the 10 reference chemicals test, the specificity 83%, sensitivity 100%. In both cases, 1-bromohexane was proved to be an irritant and 91% accuracy was achieved, reaching the OECD accuracy standard. As a result of this study, it was confirmed that the development of the skin irritation test method using the Korean type human skin model will be possible through the improvement of the washing method.

References

Jung, K. M., Lee, S. H., Ryu, Y. H. et al. (2011). A new 3D reconstituted human corneal epithelium model as an alternative method for the eye irritation test. *Toxicol In Vitro 25*, 403-410.

Jung, K. M., Lee, S. H., Jang, W. H. et al. (2014). KeraSkin™ -VM: A novel reconstructed human epidermis model for skin. *Toxicol In Vitro 28*, 742-750.

Katoh, M. and Hata, K. (2011). Refinement of Labcyte EPI-MODEL 24 skin irritation test method for adaptation to the requirements of OECD TG 439. *AATEX 16*, 111-322.

III-735

Human-based phenotypic profiling uncovers mechanisms of toxicity

Ellen Berg, Alex Folias, Adam Best, Alison O'Mahony and Sharlene Valichko

DiscoverX, Fremont, CA, United States
eberg@discoverx.com

Human-based phenotypic profiling is an attractive method for the discovery of chemical toxicity mechanisms. A large reference database was created in a standardized panel of human primary cell and co-culture model systems, the BioMAP® Diversity PLUS panel. Analysis of this data identified a biomarker signature common to skin irritants, consisting of increased prostaglandin E2 (PGE2) and decreased TNFalpha in a primary human endothelial cell co-culture model with peripheral blood monocytes stimulated with lipopolysaccharide. This signature was uncommon (30 of 3400 reference agents) and shared by 2-Chloroethyl Ethyl Sulfide, a chemical vesicant; FICZ, an Aryl Hydrocarbon Receptor (AhR) agonist; PKC, RAR/RXR, Prostaglandin EP Receptor, and Vitamin D Receptor (VDR) agonists; inhibitors of Thromboxane A2 synthetase; and lead compounds from a drug discovery program that was terminated due to skin toxicity in non-human primates.

III-760

Skin corrosion: A comparative study between the RHE equivalent and the reconstructed full thickness model

Carolina Motter Catarino, Tatiana do Nascimento Pedrosa, Paula Comune Pennacchi, Sílvia Romano de Assis, Silvia Berlanga de Morais Barros and Silvya Stuchi Maria-Engler

Skin Biology Group, Univeristy of São Paulo, São Paulo, Brazil
catarc@rpi.edu

Currently there is a strong global trend towards the development of *in vitro* models to replace the use of animals in safety evaluation tests. In Brazil, this practice is in progress and should be quickly implemented. In this study, we compare in house developed models of Reconstructed Human Epidermis (RHE) and full thickness skin (dermal and epidermal compartment) regarding their response when submitted to skin corrosion assays, based on Guide 431 (OECD). The results show that both models correctly classified the four substances tested in corrosive or non-corrosive. Furthermore, the full thickness model presented higher cell viability following exposure to the corrosive substances compared to the RHE model, which indicates better barrier function. Therefore, we have demonstrated the potential of our skin *in vitro* models to be employed as relevant and reliable test methods in research and for chemical risk assessment. Also, we have been able to highlight the contribution of the dermal compartment in increasing functionality of skin models.

References

OECD (2016). Test No. 431: In vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method, OECD Publishing, Paris.

Pedrosa, T. D. N., Catarino, C. M., Pennacchi, P. C. et al. (2017). A new reconstructed human epidermis for in vitro skin irritation testing. *Toxicol In Vitro 42*, 31-37.

Pennacchi, P. C., de Almeida, M. E., Gomes, O. L. et al. (2015). Glycated reconstructed human skin as a platform to study the pathogenesis of skin aging. *Tissue Eng Part A 21*, 2417-2425.

Innovative Models for Safety and Efficacy – Cardiac and Vascular Models

III-27

Protective effects and safety of molecular hydrogen on H9c2 cardiomyocytes model

Hideto Ariumi and Yuji Yoshiyama
Kitasato University, Tokyo, Japan
ariumih@pharm.kitasato-u.ac.jp

Opening of the infarct-related coronary artery is a valued therapeutic goal in acute myocardial infarction (AMI). However, cardiomyocytes continue to die during reperfusion. During ischemia and reperfusion, the mechanisms of cell death during this phase of AMI have been subject to debate. Although restoration of blood flow is critical, the reintroduction of molecular oxygen triggers a cytotoxic cascade during which reactive oxygen species (ROS) are generated by the mitochondria. The purpose of our study was to investigate basic mechanisms of hydrogen (H_2) gas induced cardioprotection during oxidative stress in a H9c2. H_2 gas has been demonstrated to reduce the hydroxyl radical, the most cytotoxicity of ROS, and effectively protected cells. Here, we developed a new model for evaluating that H_2 gas has the potential to act as antioxidant on H_2O_2-induced death of H9c2 cell. Treatment with H_2O_2 (0.5 mM) induced death of H9c2 cells. However, co-treatment with H_2 gas increases viability of cells. These results suggest that method is a useful alternative animal model to clarify the mechanism which H_2 gas protects H9c2 cardiomyocytes from oxidative stress.

III-33

Engineering an organotypic culture model of endocardial cushion morphogenesis to study cardiac developmental toxicity

Kyle Grode and Sid Hunter
US EPA, Research Triangle Park, NC, United States
grode.kyle@epa.gov

Congenital heart defects are the most common type of birth defect and the majority of congenital heart malformations are due to abnormal development of the valves and membranous septa (Eisenber and Markwald, 1995; Combs and Yutzey, 2009). These tissues arise from structures called endocardial cushions that develop within distinct regions of the embryonic heart. While an association between endocardial cushion defects and maternal exposure to environmental chemicals has long been recognized, the cellular events of endocardial cushion morphogenesis that are most sensitive to teratogen exposure remain unknown. Here, we summarize our efforts to engineer an organotypic culture model of endocardial cushion morphogenesis using human endothelial cells cultured on biomimetic substrates and under static or flow conditions. Our findings suggest that the incorporation of both chemical and mechanical cues is critical for recapitulating key cellular events such as endothelial-to-mesenchymal transition.

This abstract does not reflect EPA policy.

References

Combs, M. D. and Yutzey, K. E. (2009). Heart valve development. *Circ Res 105*, 408-421.
Eisenber, L. M. and Markwald, R. R. (1995). Molecular regulation of atrioventricular valvuloseptal morphogenesis. *Circ Res 77,* 1-6.

III-181

Comparative effects of nefazodone and trazodone on cardiac electrical activity in human induced pluripotent stem cell-derived cardiomyocytes

Hyang-Ae Lee[1] and Ki-Suk Kim[1,2]
[1]Predictive Model Research Center, Korea Institute of Toxicology, Daejeon, South Korea; [2]Human and Environmental Toxicology, University of Science & Technology, Daejeon, South Korea
hyangaelee@gmail.com

Although the serotonin antagonist and reuptake inhibitors (SARIs) class antidepressants, nefazodone and trazodone, are known to have fewer side effects, their actions on QT interval that may lead to cardiotoxicity have been reported. To elucidate the cellular mechanism for cardiac events induced by nefazodone or trazodone, we investigated the effects of these drugs on cardiac action potentials (APs) and ionic channels using whole-cell patch clamp technique in recently established human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and in HEK293 cells overexpressing cardiac ion channel. These drugs induced APD prolongation and early afterdepolarizations (EADs) and reduced the upstroke velocity in a dose-dependent manner. Consistent with the changes in the AP parameters, nefazodone and trazodone inhibited I_{Kr}, I_{Ks}, I_{Na}, and I_{Ca}, among them especially I_{Kr} and I_{Na}, but nefazodone had a higher inhibitory potency than trazodone. The AP assay using hiPSC-CMs could provide an integrated approach to nefazodone- and trazodone-induced repolarization and depolarization delays and its complex interactions with cardiac ion channels.

References

Lee, S., Lee, H. A., Choi, S. W. et al. (2016). Evaluation of nefazodone-induced cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes. *Toxicol Appl Pharmacol 296*, 42-53.
Lee, S., Lee, H. A., Kim, S. J. and Kim, K. S. (2016). Cellular mechanisms for trazodone-induced cardiotoxicity. *Hum Exp Toxicol 35*, 501-510.

III-312

3D vasculature-on-a-chip: A model of perfused human coronary artery endothelial microvessel for studying monocyte to endothelium adhesion under flow

Carine Poussin[1], Vincent van Duinen[2], Henriette L. Lanz[2], Angelique van den Heuvel[2], Marco van der Toorn[1], Alexandra Laurent[1], Manuel C. Peitsch[1], Julia Hoeng[1] and Jos Joore[2]

[1]Philip Morris International R&D, Neuchâtel, Switzerland; [2]Mimetas BV, Leiden, The Netherlands
carine.poussin@pmi.com

Genetic predispositions and lifestyle can promote atherogenesis that may ultimately lead to cardiovascular adverse events. To study vascular functions and disorders under flow, we envisioned the development of a perfused 3D vasculature model that mimics the *in vivo* situation. Using the OrganoPlate®, a microfluidic 3D cell culture plate supporting up to 96 tissue models, we have established culture conditions for the formation of microvessels using primary human coronary artery endothelial cells. After 4 h-treatment with various concentrations of TNF-alpha, fluorescently-labeled monocytic cells were perfused into endothelial cell microvessels and adherent monocytes were quantified from captured images, showing a concentration-dependent increase of cell adhesion to endothelial cell microvessels. In conclusion, the innovation of 3D vasculature models will open new avenues for vascular disease research and applications in pharmacology and toxicology compound screening *in vitro*.

III-553

An automated assay for the assessment of cardiac arrest in fish embryo

Marc Léonard[1], Elodie Puybareau[2], Diane Genest[1], Emilie Barbeau[1] and Hugues Talbot[2]

[1]L'Oréal Advanced Research, Aulnay sous Bois, France; [2]Université Paris Est – ESIEE, Noisy-le-Grand, France
mleonard@rd.loreal.com

Fish embryos are widely used in toxicology and ecotoxicology screening. Such tests require trained operators and the manipulation of a large number of samples. To reduce the time needed for data processing and improve objectivity, we propose to automate the mortality assessment. Here, we present an efficient image processing pipeline for heartbeat detection in Medaka (*Oryzias latipes*) embryos. We have designed a 2D acquisition device to record 1 second long video of each embryo. After a pre-processing step, the heart is identified as the area presenting the highest pixel intensity variation. As a result, based on a set of more than 2000 videos, we obtain an accuracy rate of more than 97%.

Reference

Puybareau, E., Genest, D., Barbeau, E. et al. (2017). An automated assay for the assessment of cardiac arrest in fish embryo. *Comput Biol Med 81*, 32-44.

III-668

Cytotoxicity model using human cardiomyocytes derived from pluripotent cells (iPSCs) for cardiotoxicity safety assessment

Renata Damiani[1], Flavia Valgode[1], Priscila Passos[1], Marcos Valadares[2], Diogo Biagi[2], Olga Higa[1,3] and Fabiana Medeiros[1,3]

[1]GLP Test Facility on Research and Innovation of Vitro Methods, Biosintesis Laboratory, São Paulo, Brazil; [2]Research and Innovation, PluriCell Biotech, São Paulo, Brazil; [3]Biotechnology Department, Nuclear and Energy Research Institute (IPEN/CNEN-SP), São Paulo, Brazil
renata.damiani@biosintesis.com.br

In contemporary drug development, preclinical and clinical evaluation, cardiac safety concerns arise from a variety of drug-tissue interactions, including direct myocyte toxicity. In this study, *in vitro* cytotoxic studies were conducted on GLP conditions in human cardiomyocytes derived from pluripotent cells (iPSCs). Before testing, cell population purity, above 90%, were confirmed by troponin 1 antibody biomarker. iPSCs cells were incubated with DMSO and Doxorubicin for 48 hours in a 96-well plate. Three different dyes – MTS, MTT and NR – were used to evaluate cell viability. The results showed that the iPSCs derived cardiomyocyte model was sensitive to predictive moderate and severe drug-induced cardiotoxicity. According to these results, the *in vitro* cytotoxicity model using iPSCs derived cardiomyocytes can be applied in the safety assessment of novel drug candidates as well as to identify compounds that may cause cardiotoxicity.

This study was supported by a innovative research grant PIPE program from the São Paulo Research Foundation, FAPESP.

Innovative Models for Safety and Efficacy – Models of Developmental and Reproductive Biology

III-122

Identification of potential endocrine disruptors using alternative methods according to 3R principles

Marketa Dvorakova[1,2], Kristina Kejlova[1], Hana Bendova[1], Marian Rucki[1], Adam Vavrous[1] and Dagmar Jirova[1]

[1]Centre of Toxicology and Health Safety, National Institute of Public Health, Prague, Czech Republic; [2]Third Faculty of Medicine, Charles University, Prague, Czech Republic
marketa.dvorakova@szu.cz

Endocrine disrupting substances comprise various chemicals (e.g. cyclic hydrocarbons, phenols, flavonoids, phthalates, biocides, plasticizers, surfactants, fire retardants, antimicrobials, UV filters, etc.) and are found in biological samples, consumer products, food, plastics, food contact materials, etc. *In silico* and *in vitro* screening methods were used for identification of endocrine disruption potential of certain analogues of bisphenol A, phthalates and novel antimicrobials. Presented results indicate a correlation of methods and detection of molecular interactions of certain compounds on human estrogen and androgen receptors. New chemicals being developed as replacement of compounds already regulated as endocrine disruptors should be a subject of thorough evaluation to avoid their contribution to adverse health effects. Alternative methods based on human cells and tissues are promising tools for identification of endocrine disruption in terms of systemic toxicity.

Supported by Ministry of Health, Czech Republic – conceptual development of research organization (National Institute of Public Health – NIPH, IN: 75010330).

References

Dvorakova, M., Kejlova, K., Bendova, H. et al. (2016). Alternative methods in vitro for screening of endocrine disruptors. *Neuroendocrinol Lett 37, Suppl 1*, 123-131.

Goldinger, D. M., Demierre, A. L., Zoller, O. et al. (2015). Endocrine activity of alternatives to BPA found in thermal paper in Switzerland. *Regul Toxicol Pharmacol 71*, 453-462.

Liao, C., Liu, F. and Kannan, K. (2012a). Bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol A residues. *Environ Sci Technol 46*, 6515-6522.

Molina-Molina, J. M., Amaya, E., Grimaldi, M. et al. (2013). In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. *Toxicol Appl Pharmacol 272*, 127-136.

Rogers, J. M. and Denison, M. S. (2000). Recombinant cell bioassays for endocrine disruptors: Development of a stably transfected human ovarian cell line for the detection of estrogenic and antiestrogenic chemicals. *In Vitr Mol Toxicol 13*, 67-82.

III-184

Quantitative image-based assessment of morphological features in zebrafish to improve developmental toxicity assessment

Elisabet Teixido[1], Tobias Kießling[1,2] and Stefan Scholz[1]

[1]Department of Bioanalytical Ecotoxicology, Helmholtz-Zentrum für Umweltforschung GmbH – UFZ, Leipzig, Germany; [2]Scientific Software Solutions, Leipzig, Germany
elisabet.teixido@ufz.de

Developmental toxicological studies are usually required in both a rodent and a non-rodent species. Testing in a second species will not be waived until it is demonstrated that other approaches would reveal the same level of information on potential developmental toxicity. Zebrafish has become a key alternative model but efforts are needed to improve its performance. Hence, an automated image-based quantification of morphological features in toxicant-treated zebrafish embryos was developed. Images of zebrafish larvae were collected using the VAST Bioimager to handle and proper orient embryos. To detect the morphological features a standalone software named FishInspector was developed based on MATLAB scripts. For each endpoint concentration-response curves were derived to rank the toxicity, cluster responses and potentially derive diagnostic signatures. Some of the substances displayed a high toxic ratio, suggesting a specific interaction with embryonic development. In some cases, quantitative endpoints were more sensitive than cumulative manual analysis. This approach contributes to understand the predictive capacity of the zebrafish embryo assays and strategies to improve it.

III-374

The role of ABCG2 in mouse embryonic stem cell development

Maria Hoopes[1], Mitch Rosen[1], Kelly Chandler[2], S. Charla[1], Harriette Nichols[1] and Edward S. Hunter III[1]

[1]ORD/ISTD/SBB, US EPA, Research Triangle Park, NC, United States; [2]Office of Policy, Planning, and Evaluation, NIEHS, Research Triangle Park, NC, United States
Hoopes.Maria@epa.gov

ABCG2 is a commonly studied efflux transporter. The role of ABCG2 during early development, however, is not clear. Previous studies suggested that regulation of ABCG2 could be associated with altered differentiation in mouse embryonic stem cells (mESC) via altered redox status. ABCG2 activity was found to expand during early mESC differentiation. Pharmacological inhibition of ABCG2 was used, with and without xenobiotic exposure, to evaluate differentiation. Inhibition of ABCG2 in combination with chemicals predicted by ToxCast to regulate ABCG2 did not modify toxicity. Moreover, while inhibition of ABCG2 increased the toxicity of certain chemotherapeutics, it did not shift the toxicity of oxidative stressors. Hence, ABCG2 serves a protective role during development, although it's role in regulating redox status is unclear. The hypothesis that regulation of ABCG2 by xenobiotics may be related to altered differentiation could not be supported.

This abstract does not reflect EPA policy.

III-423

A novel *in silico* model for systematic prediction of developmental toxicity

Sid Hunter[1], Kyle Grode[1], Mitch Rosen[1], Dustin Kapraun[1], Nancy Baker[2], Richard Judson[1] and Thomas Knudsen[1]

[1]US EPA, Research Triangle Park, NC, United States; [2]Leidos, Research Triangle Park, NC, United States

hunter.sid@epa.gov

Animal-based methods are the standard approach to assess developmental toxicity. Mechanism-based *in vitro* assays and *in silico* predictive tools are desired alternatives. We used 4 elements to develop a novel *in silico* predictive model: 1. Mouse Genome Informatics database to compile all gene signals linked to morphological development; 2. Panther Gene Ontology to organize genes into pathway-based classification models; 3. ToxCast *in vitro* bioactivity database to identify chemical-gene target pairs for > 1000 chemicals and concentration response profiles; 4. Fetal high-throughput toxicokinetic model to estimate human fetal serum concentrations following a 1 mg/kg/day oral exposure during gestational week 14. The classification model for cardiovascular development identified 88 (of 416 chemicals) potential cardiac disrupters at realistic maternal exposures. This *in silico* approach can be used to rank or prioritize untested environmental chemicals for further action.

This abstract does not present US EPA policy.

III-436

A cell panel-based real-time cellular analysis method for detection and differentiation of endocrine disruptors

Diana Guimet, Can Jin, David Santa Ana and Yama Abassi

ACEA Biosciences, Inc, San Diego, CA, United States

dguimet@aceabio.com

An *in vitro* method is developed utilizing mammalian cell panel to identify endocrine disruptor chemicals (EDCs) affecting estrogen receptor (ER), androgen receptor (AR) and thyroid hormone receptor (THR). Modulation of the pathways leads to changes in cell number and morphology which are detected by gold microelectrodes embedded in the bottom of the well of a specialized microtitre plate. Each of the tested cell lines displayed different sensitivities and kinetic response profiles. Human breast cancer cell line T47D sensitively and selectively detects ER and AR agonists, with distinct kinetic response profile for each category. The response profiles can be specifically inhibited by antagonists targeting same receptors. Rat pituitary tumor cell line GH3 showed sensitive proliferative response to TR agonists. Human prostate cancer cell LNCAP line is the most sensitive among the cell panel toward AR modulators. In summary, this method collectively uses mammalian cells with different tissue and species origins, to detect and differentiate 3 distinctive classes of EDCs within a single cellular assay.

References

Atienza, J. M. et al. (2005). Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays. *J Biomol Screen 10*, 795-805.

Grimaldi, M. et al. (2015). Reporter cell lines for the characterization of the interactions between human nuclear receptors and endocrine disruptors. *Front Endocrinol 11*, 62. doi:10.3389/fendo.2015.00062

Rotroff, D. M. et al. (2013). Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T47D human ductal carcinoma cells. *Chem Res Toxicol 26*, 1097-1107.

U.S. Environmental Protection Agency (2014). Endocrine Disruptor Screening Program Comprehensive Management Plan. 1-30.

Yang, O. et al. (2015). Endocrine-disrupting chemicals: Review of toxicological mechanisms using molecular pathway analysis. *Eur J Cancer Prev 20*, 2288-3657.

III-437

Using artificial intelligence and high-throughput hormone measurements to predict chemical effects on steroidogenesis

Agnes Karmaus[1] and Lyle Burgoon[2]

[1]ILS, Inc., Morrisville, NC, United States; [2]US Army Engineer Research and Development Center, Research Triangle Park, NC, United States

akarmaus@ils-inc.com

We have constructed a Bayesian network to predict which enzymes in the steroidogenesis pathway may be impacted by chemical exposure. Briefly, steroid hormones and enzymes were encoded as nodes with edges between nodes representing conditional probabilities. For instance, two nodes lead to progesterone, such that the probability of progesterone production is conditional on probabilities for both pregnenolone and HSD3B1 activity being present. Using this Bayesian network, we can query the likelihood of any represented enzyme in the steroidogenesis pathway being active or inactive. Data for hormone levels were retrieved from the ToxCast high-throughput H295R assay for input. Based on these data where 936 chemicals altered the levels of ≥ 1 hormone, and a subset of 227 chemicals altered the levels of ≥ 4 hormones, our model predicted specific enzyme inhibition for 178 chemicals identifying putative novel targets for chemicals and possible mechanisms underlying steroidogenesis disruption.

III-512

Lifestage-specific organotypic modeling platform for adverse outcome pathways of male reproductive and developmental processes

Rachel M. Shaffer, Carly S. Wilder, Catherine Ryu, Sun Young Park, Sungwoo Hong, Collin C. White, Tomomi Workman, William Griffith and Elaine M. Faustman

Environmental and Occupational Health Sciences, UW Seattle School of Public Health, Seattle, WA, United States

rms14@uw.edu

Development of the male reproductive system is vulnerable to disruption from chemical exposure, particularly during critical windows of susceptibility. We have established a novel 3D mouse testicular co-culture *in vitro* system to evaluate the effects of exposures during postnatal days (PND) 9-25. This time period has been identified as a critical window in the mouse testis through transcriptomic analysis of publicly available data and a developmental timeline based on a literature search. We analyzed long-term viability, testosterone production and morphology up to 16 days in culture to characterize baseline features of the system. Sertoli, Leydig and spermatogonial germ cells were identified using cell-type specific markers of proliferation and differentiation with Western blots and immunofluorescence. There was a biphasic pattern of testosterone concentration in culture, which is expected during this period because of the transition from fetal to adult Leydig cell populations. A known testicular toxicant, cadmium, was used to evaluate effects of chemical exposure during this critical window of susceptibility. We exposed the co-culture system to cadmium (2.5, 5 and 10 μM concentrations) on days *in vitro* (DIV) 2, 6, and 15 and characterized testosterone production, cytotoxicity, cell viability, and protein expression after 24 hours of exposure. Initial studies have observed dose dependent cytotoxicity and differential susceptibility based on time of exposure. These lifestage-specific quantitative results can be interpreted within the context of an Adverse Outcome Pathway (AOP) and demonstrate the potential of our model to capture adverse outcomes in proliferation, steroid regulation and spermatogenesis pathways of male reproductive development. Additionally, comparison of 3D results with pilot "testis-on-a-chip" model results provides context for cross-platform evaluations.

III-544

A benchmark study of known endocrine disruptor compounds combining standard environmental and human-cell based *in vitro* methods

Anne Riu[1], Sylvie Emery[1], Reine Note[1], Noémie De Croze[1], Marc Léonard[1], Patrick Balaguer[2] and Silvia Teissier[1]

[1]L'Oréal, R&I, Aulnay sous Bois, France; [2]IRCM, INSERM, Montpellier, France

ariu@rd.loreal.com

Even if no clear identification criteria have been decided yet about Endocrine Disruptor Compounds in the European Union, concerns have been raised by scientists, media and consumers on certain chemicals which might be disrupting the endocrine systems and wildlife. Efforts have been made to build an evaluation toolbox covering human and environmental safety assessment using *in silico*, *in vitro* and fish-embryo models for early screening purposes. A set of 24 chemicals from various origin and well known for their estrogen-like properties *in vitro* were evaluated with assays currently available for the screening of endocrine active compounds. The focus of this study was on the estrogen receptor binding pathway which is one of the most documented so far. The comparison of the results demonstrated the complementarity of these assays and confirmed that one single assay cannot be used in a standalone manner to address a specific endocrine pathway.

III-545

Improvement of the value of an *in vitro* endocrine disruption assay by incorporating a metabolizing system

Anne Riu, Sylvie Emery, Guilaume Lereaux, Eric Arbey, Sébastien Grégoire, Joan Eilstein, Silvia Teissier and Reine Note

L'Oréal, R&I, Aulnay sous Bois, France

ariu@rd.loreal.com

It is recognized that metabolically competent *in vitro* cell-based assays are required for the efficacy and safety assessment of xenobiotics. Regulatory bodies emphasized the need for developing appropriate metabolically competent *in vitro* assays mainly for the assessment of potential endocrine active substances (Jacobs et al., 2013). To comply with these recommendations and enrich our testing strategy, a method was developed incorporating a metabolic system (liver and skin S9 fractions) into the current transactivation assays for estrogenic and androgenic activities assessment. The method was developed using well-known compounds such as Methoxychl or, Bisphenol A, Vinclozolin. In the presence of S9 fraction with their phase I and II cofactors, toxic metabolites and/or metabolically detoxified compounds were observed. The feasibility and the relevance of the incorporation of metabolic systems into *in vitro* assays were demonstrated for optimizing the characterization of a potential endocrine activity of new compounds.

Reference

Jacobs, M. N., Laws, S. C., Willett, K. et al. (2013). In vitro metabolism and bioavailability tests for endocrine active substances: What is needed next for regulatory purposes. *ALTEX 30*, 331-351.

III-617

New methods for the evaluation of endocrine disruptors on human placental model

Patrice Rat[1], Mélody Dutot[1], Patrick Vitaux[2], Elodie Olivier[1] and Anaïs Wakx[1]

[1]Toxicology Laboratory – Faculty of Pharmacy of Paris Descartes University, Paris, France; [2]Bertin Pharma, Montigny-le-Bretonneux, France

anais.wakx@free.fr

The World Health Organization (WHO) describes endocrine disruptors (EDs) as substances that alter functions of the endocrine system and cause adverse health effects in an intact organism or its progeny. Currently, the most used *in vitro* assays for high throughput screening are estrogen receptor binding and estrogen receptor transcriptional activation assays. As these tests only focus on alterations of the endocrine system, they are not sufficient to match WHO's definition. Our aim was to develop a simple and fast assay to detect both endocrine system alteration and adverse health effects. We worked on a human placental cell line that secretes hormones and expresses proteins involved in adverse health effects to highlight endocrine disruption after a short exposure time. Our cell-based assay seems to reveal endocrine toxicity and could be proposed to normalization agencies (CEN/ISO) to evaluate potential EDs.

III-682

Using SAR and high throughput data to fill data gaps for developmental and reproductive toxicity hazards

Sara Pacheco-Shubin[1], Dan Skall[2] and Thomas Lewandowski[1,3]

[1]Gradient, Seattle, WA, United States; [2]Gradient, Cambridge, MA, United States; [3]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States

sshubin@gradientcorp.com

To assess the utility of structural-activity relationship (SAR) and high-throughput data for read across, we examined zebrafish ova assay and SAR data for 93 chemicals previously assessed for developmental/reproductive toxicity (DART) using standard animal tests. In our analysis, 62% of the chemicals were true positives (positive in both the zebrafish assay and standard tests) whereas 54% were false positives (positive in the zebrafish assay but negative in standard tests). Most false positives were non-DART agents in standard tests due to maternal toxicity, which is not addressed in the zebrafish assay. False negative chemicals (38%) were suspected DART agents due to endpoints not investigated in the zebrafish assay (e.g., male reproductive toxicity). There was much less concordance between the animal and SAR predictions; only 19% of DART agents identified via standard studies were SAR-positive. These results not only support the potential utility of the zebrafish ova assay for filling DART data gaps, but also highlight the need for multiple reliable screening assays to cover the wide array of developmental and reproductive endpoints *in vivo*.

Innovative Models for Safety and Efficacy – Liver

III-9

Hepatotoxicity evaluation using spheroid coculture system of primary human hepatocyte and human Kupffer cell

Hiroko Hitotsumachi[1], Tomoaki Hitotsumachi[2], Hiroaki Oka[2], Fumio Morita[2] and Kazuo Hakoi[2]

[1]Toxicology Laboratory, Taiho Pharmaceutical Co., Ltd, Tokyo, Japan; [2]Taiho Pharmaceutical Co., Ltd, Tokushima, Japan
h-kitayama@taiho.co.jp

Drug-induced hepatotoxicity is serious adverse event in clinical study. The use of animals in toxicology studies is controversial due to ethical and interspecies differences. 3D hepatocyte spheroid models have been developed. The similarity to the *in vivo* situation is valuable feature for an *in vitro* model simulating *in vivo* conditions. To estimate the utility for coculture spheroid system using primary human hepatocyte (PHH) and Kupffer cell (KC), we confirmed several endpoints using coculture spheroid treated with hepatotoxicity-induced drugs. Spheroid showed increased irregular morphology and varied intracellular responses dose-dependently by each drug. We identified KC and distinct findings histologically. Biomarker levels of supernatant were varied by each drug and their exposure period. We revealed that hepatotoxicity could be detected by measuring morphology, intracellular responses and biomarker levels of spheroid cocultured with PHH and KC. Furthermore, combination of evaluation items was valuable method for detecting hepatotoxicity *in vitro*.

III-158

Microplate-based hierarchically-layered coculture with enhanced oxygenation as a new physiological liver tissue

Yasuyuki Sakai[1,2,3], Marie Shinohara[2], Matheu Danoy[3,4], Wenjin Xiao[2], Yukimi Kobayashi[5], Kikuo Komori[1], Katsuto Otake[5] and Vincent Senez[4]

[1]Department of Chemical System Engineering, University of Tokyo, Tokyo, Japan; [2]International Research Center on Integrative Biomedical Systems, Institute of Industrial Science, University of Tokyo, Tokyo, Japan; [3]LIMMS/CNRS, UMI2820, Institute of Industrial Science, University of Tokyo, Tokyo, Japan; [4]IEMN, University Lille, CNRS, Lille, France; [5]Tokyo University of Science, Tokyo, Japan
sakaiyas@iis.u-tokyo.ac.jp

To develop better physiological liver tissue *in vitro*, parenchymal hepatocytes should be cocultured with non-parenchymal cell populations in an *in vivo*-like hierarchical manner. Here, we successfully organized double- or triple-layered coculture liver tissue in oxygen-permeable microplates. Combination of rat hepatocytes with an endothelial cell line resulted in enriched ECMs between the two cell layers, leading to enhanced hepatic functions as well as tissue polarity (Xiao et al., 2015). Combination of hepatocytes with both an endothelial cell line and a stellate cell line showed less responses against inflammatory stimulation (Danoy et al., 2017); stellate cells initially produced more ECMs with enhanced oxygenation, but later the formed tissue even showed a less-inflammatory status, suggesting successful formation of quiescent tissues as opposed to usual cultured tissue models. As such, oxygen-permeable plate-based hierarchical coculture of liver cell populations can provide a new liver tissue model useful for both pharmacological/toxicological assays and liver disease models *in vitro*.

References

Danoy, M., Shinohara, M., Rizki-Safitri, A. et al. (2017). Alteration of pancreatic carcinoma and promyeloblastic cell adhesion in liver microvasculature by co-culture of hepatocytes, hepatic stellate cells and endothelial cells in a physiologically-relevant model. *Integr Biol 9*, 350-361.

Xiao, W., Perry, G., Komori, K. and Sakai, Y. (2015). New physiologically-relevant liver tissue model based on hierarchically cocultured primary rat hepatocytes with liver endothelial cells. *Integr Biol 7,* 1412-1422.

III-186

The importance of sufficient oxygen supply to the cell layer by using oxygen permeable membrane for human hepatocytes culture

Keiichi Kimura[1], Marie Shinohara[2], Wenjin Xiao[2] and Yasuyuki Sakai[1,2]

[1]School of Engineering, University of Tokyo, Bunkyo-ku, Japan; [2]Institute of Industrial Science, University of Tokyo, Tokyo, Japan
k_kimura@chemsys.t.u-tokyo.ac.jp

Oxygen supply to cultured cell layers in conventional polystyrene (PS) plates cannot satisfy the oxygen demand of hepatocytes. This results in the rapid decline of their metabolic capacities *in vitro*. One solution to achieve sufficient oxygen supply is applying a polydimethylsiloxane (PDMS) membrane, which has high oxygen permeability, to the bottom surface of culture. In this study, we cultured human hepatocytes (HHs) isolated from humanized mice (PXB-mice) in both PDMS and PS plates. In the PDMS culture, HHs showed about three times higher oxygen consumption when compared to those in PS plates and this cellular consumption observed in PDMS plates was close to in situ perfused liver values. Moreover, HHs in PDMS plates showed higher albumin secretion and CYP3A4 activity until day 14 when cultured with Matrigel supplementation or co-cultured with 3T3 cells. Thus, sufficient oxygen supply with PDMS membrane is an effective culture method for improving hepatic function of HHs *in vitro*.

III-305

HepaRG™ cells combined with a genotoxin-specific qPCR array as a human-relevant and rapid tool for improved hazard assessment

Vera Rogiers[1], Birgit Mertens[2], Luc Verschaeve[2], Dimiter Milushev[3], Nancy H. C. Roosens[2], Sigrid C. J. De Keersmaecker[2], Tatyana Y. Doktorova[2] and Gamze Ates[1]

[1]Vrije Universiteit Brussel, Brussels, Belgium; [2]Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium; [3]Super Stellar Solutions, Oudergem, Brussels, Belgium
vrogiers@vub.ac.be

Regulatory *in vitro* batteries for genotoxicity testing suffer from a high number of misleading positives. This has impact on the development of new cosmetic ingredients in the EU, as afflicted compounds have to be abandoned because of the testing ban. By using a toxicogenomics approach, we have developed a gene classifier based on the transcriptomic changes induced by 12 genotoxic – covering most known mechanisms – and 12 non-genotoxic reference compounds. Subsequently the gene classifier was translated into an easy-to-handle qPCR array. To assess the predictivity of the developed tool, 5 known positive, 5 known negative and 2 equivocal test compounds for genotoxicity were evaluated and an accuracy of 100% was obtained, when equivocal results were considered as positive. Therefore, the combination of metabolically competent human HepaRG™ cells with a genotoxin-specific qPCR array can be proposed for genotoxic hazard assessment, particularly as a part of a weight-of-evidence approach.

III-442

The advantages of *in vitro* 3D liver model in liver toxicity evaluation

Mo Dan

National Institutes for Food and Drug Control, Beijing, People's Republic of China
danmo543@163.com

A 3D multicellular sphere model of HepaRG cells was constructed and albumin, urea levels and hepatic enzyme activities were tested for 14 days. The hepatotoxicity of isoniazid and amiodarone hydrochloride were studied using the 3D liver model and 2D cell culture. After 4 days in culture cells, HepaRG cells formed a compact spheroid. Albumin and urea levels and the CYP3A4 activity were significantly higher than 2D from day7 to day14. The IC_{50} of amiodarone hydrochloride in 2D and 3D models were 50 and 100 μM, respectively. The LDH level in 3D model increased with a dose-dependent manner. Isoniazid (1-1000 μM) had no hepatocyte toxicity in 2D model, while the IC_{50} in 3D model was 700 μM. The LDH level also showed significant increases with a time and dose dependent manner. *In vitro* 3D hanging-drop liver model demonstrated good liver functions and has high hepatic drug-metabolizing enzyme activities. Compared with 2D, 3D liver model can accurately evaluate the liver toxicity of drugs. Our results demonstrated the importance of long lasting *in vitro* liver culture models that allow repeated drug-treatments for detection of *in vivo*-relevant adverse drug effects.

III-443

Protective effects of diammoniumglycyrrhizinate on liver toxicity induced by triptolide

Zuogang Li

National Institutes for Food and Drug Control, Beijing, People's Republic of China
lizg428@nifdc.org.cn

Objective: To study the protective effects of diammoniumglycyrrhizinate (DG) on liver toxicity induced by triptolide (TP).

Methods: TP induced ROS in HepG2 and the protective effect of DG was studied using high content analysis (HCA). ADMET Predictor was used to predict the effect of CYP3A4 on TP metabolism. CYP3A4 inhibitor and inducer were used to verify whether DG upregulated CYP3A4 activity to protect TP induced liver toxicity.

Results: The IC_{50} on HepG2 after 1, 2 and 4 TP treatments were 30, 20 and 15 $\mu mol \cdot L^{-1}$, respectively. After 48 h pretreatment of DG (10, 100 and 1000 $\mu mol \cdot L^{-1}$), the IC_{50} on HepG2 (1 h TP treatment) were increased to 60, 85, 89 $\mu mol \cdot L^{-1}$, respectively. TP induced ROS on HepG2 demonstrated a dose-dependent manner using HCA. After DG pretreatment, TP induced ROS was significantly decreased. ADMET Predictor™ predicted that TP inhibits CYP3A4 activity. ROS-induce ability of TP can be inhibited after 48 h CYP3A4 inducer or DG pretreatment.

Conclusion: DG showed significantly protective effects on the TP induced liver toxicity. CYP3A4 may play an important role on DG protective effects against TP induced liver toxicity.

III-462

Development of *in vitro* hepatotoxicity assay system focusing the drug induced liver injury (DILI) mechanism

Morihiko Hirota[1], Sekine Shuichi[2], Takumi Nukaga[2], Moemi Kawaguchi[2], Takeshi Susukida[2], Akinori Takemura[2], Cong Liu[2], Akiko Tamura[1], Shiho Oeda[1], Nana Kuwata[1], Kousei Ito[2] and Hirokazu Kouzuki[1]

[1]Shiseido Global Innovation Center, Yokohama-shi, Kanagawa, Japan; [2]Graduate school of Pharmaceutical Sciences, Chiba University, Chiba, Japan
morihiko.hirota@to.shiseido.co.jp

Purpose: Systemic toxicity is one of difficult endpoints to assess safety using *in vitro*/*in silico* model. In this study, we focused hepatotoxicity, which is major target organ in systemic toxicity and tried to develop corresponding 4 *in vitro* tests.

Methods: Mitochondrial dysfunction or steatohepatitis were evaluated by cytotoxicity or lipid accumulation in HepG2 under crabtree-effect circumventing culture condition. Cholestasis was evaluated by cytotoxicity induced by bile acids accumulation of in human sandwich cultured hepatocytes. Inhibition of bile canalicular formation was evaluated by immune-fluorescence analysis in sandwich cultured HepG2.

Results and discussion: When we tried score-based data integration of 4 tests data of 26 chemicals, the accuracy for 3 category potency based on drug induced liver injury (DILI) score was about 70%. These data showed that the integration of mechanism based *in vitro* assays might be useful for weight of evidence assessment for hepatotoxicity.

III-542

Development of a 3D *in vitro* model for the assessment of repeated dose hepatotoxicity

Anne Riu, Sylvie Emery, Hélène Riesterer, Reine Note, Silvia Teissier and José Cotovio

L'Oréal, R&I, Aulnay sous Bois, France
ariu@rd.loreal.com

The development of innovative *in vitro* methods able to reproduce the physiological functionality of specific human organs became essential and must continue in order to face the current challenges for risk assessment purposes, since the animal ban testing. Liver was shown to be the most targeted organ by cosmetics ingredients in repeated dose studies *in vivo* (1). A 3D liver spheroid assay based on HepG2 cells was developed and maintained up to 10 days to assess chronic hepatotoxicity of new cosmetic ingredients. The viability and the improving hepatic functionalities of the system such as metabolic activities and albumin secretion were monitored over a 10-day period. Well known hepatotoxicants such as acetaminophen, amiodarone, valproate, etc., were used to validate the model and specific biomarkers were selected in order to predict early hepatotoxic effects before reaching cell mortality. A larger set of compounds was evaluated to expand the applicability domain of this model. This cost-effective model could be promising for a further screening of new cosmetic ingredients for long-term hepatotoxicity effect.

Reference

Vinken, M. et al. (2012). Screening of repeated dose toxicity data present in SCC(NF)P/SCCS safety evaluations of cosmetic ingredients. *Arch Toxicol 86*, 405-412.

III-549

A mechanism-based simulation model of hepatic toxicity to predict cell death dynamics through mitochondrial dysfunction upon long-term exposure to drug

Anne Riu[1], Alina Crudu[1], Kazuhiro Fujita[2], Gladys Ouedraogo[1], Yukiko Matsuoka[2], Shungo Miyabe[3], Itaru Suzuki[3], Silvia Teissier[1], Bernard Fromenty[4], Samik Ghosh[2], Reine Note[1] and Hiroaki Kitano[2]

[1]L'Oréal, R&I, Aulnay sous Bois, France; [2]The Systems Biology Institute, Tokyo, Japan; [3]L'Oréal R&I, Japan; [4]Unité INSERM UMR991, Rennes, France
ariu@rd.loreal.com

Mechanism-based prediction model of toxicity upon chemical exposure is becoming relevant, particularly in the context of the ban on animal testing in the cosmetic field. While adverse outcome pathways (AOP) elucidate the knowledge-base for mechanisms of toxicity, introduction of dynamic simulation models gives a better quantitative understanding of dynamics of toxic responses by using systems biology approaches. A simplified mechanistic model of hepatic toxicity through mitochondrial dysfunction was built based on AOP and curated literature. The dynamics of the simulation model were constrained by fitting parameters computationally in the model to the dynamics of measured mitochondrial outcomes observed in HepaRG cells upon a 12-daychronic exposure to known hepatotoxic drugs, such as amiodarone, valproate, acetaminophen and troglitazone. The developed model successfully captured underlying mechanisms of mitochondrial dysfunction *in vitro* such as inhibition of beta-oxidation and respiratory chain and generation of reactive species.

III-658

Use of mass spectrometry imaging and a full thickness 3D skin equivalent (EpiDermFT) for evaluation of percutaneous absorption

Michael Bachelor[1], Erin Seeley[2], Callee Walsh[2], Alex Armento[1], Patrick Hayden[1], Mitchell Klausner[1] and Haddon Goodman[2]

[1]MatTek Corporation, Ashland, MA, United States; [2]Protea Biosciences, Morgantown, WV, United States
mbachelor@mattek.com

Human skin equivalents are useful tools for evaluating percutaneous permeation/absorption and quantities of active that traverse the skin, remain on the skin surface or are retained within the skin. Incorporating mass spectrometry imaging (MSI) allows for localization of compounds along with their relative concentrations, and determining how much compound reaches its target location. In this study, an OTC retinol complex was applied to EpiDermFT, a full thickness skin equivalent, to evaluate permeation and localization. Retinol and formulation components in the epidermal or dermal layer were successfully imaged by MSI. EpiDermFT in combination with MSI can be used in development of cosmetics and pharmaceuticals to better understand where actives localize following topical application. As the EpiDermFT model has demonstrated drug metabolizing capabilities, these technologies can be used to gain insight into localization of drug metabolites and other biomolecules following treatment.

III-712

Assessment of an oxidative stress gene reporter system in an immortalized glutathione-deficient mouse hepatocyte cell line

Terrance Kavanagh[1], Dianne Botta[1], Collin White[1], Christopher Schaupp[1], Kirk Van Ness[2], Edward Kelly[2] and David Eaton[1]

[1]DEOHS, University of Washington, Seattle, WA, United States; [2]Pharmaceutics, University of Washington, Seattle, WA, United States

tjkav@uw.edu

Glutathione (GSH) is an important cell antioxidant. Glutamate cysteine ligase (the rate-limiting enzyme for GSH synthesis) is composed of catalytic (GCLC) and modifier (GCLM) subunits, and are regulated by the redox responsive Nrf2 transcription factor. We made $Gclm^{-/-}$ mice by replacing Exon 1 with a b-galactosidase/neomycin phosphotransferase fusion gene. DDAOG is a substrate that can be used to assess b-gal activity, and DDAO fluorescence thus serves as a reporter of Nrf2-driven Gclm gene expression in these mice. We made immortalized hepatocyte cell lines from $Gclm^{-/-}$ and $Gclm^{+/+}$ mice (IM/KO and IM/WT cells). These cells were exposed to the oxidant hydroquinone, which caused a dose related depletion of GSH and an increase in cell death and in b-gal activity in IM/KO cells but not in IM/WT cells. These results show the utility of this system for investigating chemically induced oxidative stress, glutathione-dependent cell viability, and Nrf-2 dependent gene transcriptional activity *in vitro*, and reduce the use of mice in screening chemicals for oxidative stress.

Support: EPA grant RD-83573801, NSF Grant CHE-1339637, NIH Grants P30ES007033, T32AG000057.

III-718

High throughput microscopy of adaptive stress responses in unraveling and predicting DILI

Bob van de Water

Leiden University, Leiden, The Netherlands

b.water@lacdr.leidenuniv.nl

The activation of adaptive stress response pathways is a key event in drug-induced cell injury. Toxicogenomics has established the key stress pathways that are involved in liver injury. We have established GFP-based adaptive stress response reporters in HepG2 cells based on bacterial artificial chromosome transgenome technology. These reporters allow the analysis of the dynamics of stress pathway activation at the single cell level in high throughput microscopy based assays. Our reporters cover, amongst other, oxidative stress, DNA damage, unfolded protein response and heat shock responses. We have systematically assessed the application of these reporters for the prediction of DILI in both 2D monolayer and 3D spheroid systems using automated imaging and compared our results to data in primary human hepatocytes based on legacy data. We have compared the use of these reporters for single dosing and repeated dosing regimens with either end-point measurements or taking advantage of live cell imaging. Also, concentration ranges that are based on available maximal human *in vivo* plasma concentration for each individual compound or applying the same concentration range for all compounds has been tested. Overall the data indicate that different DILI drugs activate different stress response reporters and that these reporters contribute to an improved mechanism-based assessment of DILI liability.

Innovative Models for Safety and Efficacy – Renal

III-339

A robust *in vitro* islet model: Long-lived, standardized and glucose-responsive human islet microtissues

Lei He, Aparna Neelakandhan, Adelinn Biernath, Olivier Frey and Burcak Yesildag

InSphero AG, Schlieren, Switzerland
lei.he@insphero.com

Inherent heterogeneity in pancreatic islet size, cellular composition and function as well as the short *ex vivo* lifespan of both rodent and human islets, pose a significant challenge for their *in vitro* use and result in waste of precious primary material. To address this issue, we developed a standardized 3D islet model that is uniform in size, composition and architecture and cultured in 96-well-plates in a single islet per well format to enable high-throughput data acquisition with low intra-assay variability. Our model displayed reproducible and robust glucose-regulated insulin and glucagon secretion across donors for 28 days in culture. In perfusion experiments, increase in glucose induced biphasic and pulsatile insulin secretion closely mimicking dynamic *in vivo* responses. Quantification of relative proportion of endocrine cells reflected fractions found within the human pancreas. Long-term exposure to metabolic stressors (high glucose and free fatty acids) reversibly impaired islet function. Our results demonstrate that this model is suitable for high-throughput and long-term study of islet function and regeneration in health and disease.

III-486

Development of assays for understanding the role of mitochondrial toxicity in chemical induced renal injury

Giada Carta, Alice Limonciel, Georg Kern, Thomas Haller, Anja Wilmes and Paul Jennings

Physiology and Medical Physics, Medical University of Innsbruck, Innsbruck, Austria
giada.carta@i-med.ac.at

Primary or secondary mitochondrial injury is a factor in the nephrotoxicity of many chemicals. As part of a wider mitochondrial case study in the EU-ToxRisk project we focused on the optimisation of methods for the detection of mitochondrial toxicity.

The human renal proximal tubular cell line RPTEC/TERT1 were cultured in 96 well plates and differentiated in defined medium. Cells were exposed to 6 concentrations of 22 respiratory chain complex inhibitors in medium in either 5.5 mM glucose or 5.5 mM galactose for 24 h. Mitochondrial function was investigated studying resazurin reduction, glycolysis (supernatant glucose and lactate), and mitochondrial specific dyes (JC-1, rhodamine 123, Calcein/cobalt and mitotracker red).

JC-1 was the preferred assay for mitochondrial effects. Decreased resazurin and increased lactate were concomitant with decreased JC-1. Complex I inhibitors had the largest impact on these endpoints. The results, optimised assays and future perspectives are presented.

III-612

Polycationic antibiotic exposure validates clinical findings using a 3D microphysiological kidney-on-a-chip

Elijah Weber[1], Martti Vaara[2], Timo Vaara[2], Thomas Neumann[3], Jonathan Himmelfarb[4] and Edward Kelly[1]

[1]Pharmaceutics, University of Washington, Seattle, WA, United States; [2]Northern Antibiotics Ltd, Helsinki, Finland; [3]Nortis Inc., Seattle, WA, United States; [4]Kidney Research Institute, Seattle, WA, United States
ejweber@uw.edu

The renal proximal tubule is a primary site of drug-induced injury which is highly influenced by intracellular accumulation from facilitated transport-mediated processes. To further understand this complicated process of accumulation and injury we have developed a 3D microphysiological system (MPS). As polymyxins remain as a last-line highly effective therapy when treating drug-resistant bacteria, the frequency of drug-induced injury is significant (~30%) despite mechanisms of polymyxin-induced nephrotoxicity continuing to remain unknown. Our goal is to accurately model and assess the safety of polymyxin exposure in the kidney MPS to elucidate mechanisms of toxicity. To date, our findings have shown that the kidney MPS is sensitive to drug-induced toxicity, showing significant induction of urinary biomarkers (kidney injury molecule-1), cell-associated biomarker (heme-oxygenase-1) induction, and significant transcriptional changes following acute (48 h) exposure to 50 μM polymyxin B. Furthermore, a rigorous safety assessment will be achieved using new polymyxin-analogues shown to have improved preclinical safety.

III-649

Microphysiological system assessment of nephrotoxicity of $CdCl_2$ and quantum dots with a cadmium/selenium core

Kirk Van Ness[1], Terry Kavanagh[2] and Edward Kelly[1]

[1]Pharmaceutics, University of Washington, Seattle, WA, United States; [2]Env. and Occ. Health Sciences, University of Washington, Seattle, WA, United States
kpvn@uw.edu

Electronic industry workers exposed to quantum dots (QD) with cadmium/selenium cores may be at risk of nephrotoxicity due to cadmium's propensity to target renal tissue. To investigate whether potential nephrotoxicity could occur with QD exposure, we exposed human proximal tubule epithelial cells (PTECs) to a QD with a CdSe/ZnS core/shell in 2D and 3D microphysiological (MPS) formats. The QD formulation we analyzed was positively charged and contained a polydiallydimethylammounium chloride coating. PTECs seeded into MPS devices were exposed for 48 hours to medium, 25 μM $CdCl_2$ (positive control) or 2.5 nM of QD. In addition, we exposed PTECs to 0, 0.25, 2.5 and 25 μM $CdCl_2$ and collected RNA after 48 hours of exposure for RNA-seq analyses. MPS effluents were collected at 24 and 48 hours and analyzed for kidney injury biomarkers. RNAseq data from the $CdCl_2$ dosimetry experiment have shown that $CdCl_2$ induces oxidative stress, metallothioneins, and heat shock protein transcripts.

Innovative Models for Safety and Efficacy – Genotoxicity and Cancer Models

III-13

Extrapolation of *in vitro* mutagenicity alerts to the *in vivo* endpoint in Derek Nexus

Rachael Tennant[1], Steven Canipa[1], Alex Cayley[1], Will Drewe[1], Sebastien Guense[1], Susanne Stalford[1], Richard Williams[1], Kenichi Masumura[2], Takeshi Morita[2] and Masamitsu Honma[2]

[1]Lhasa Limited, Leeds, United Kingdom; [2]National Institute of Health Sciences, Tokyo, Japan

rachael.tennant@lhasalimited.org

As part of the hazard and risk assessment of chemicals, it is important to assess the ability of a chemical to induce mutations *in vivo*. This is based not only on the causal link between mutations and cancer but also on the potential for mutations to induce other, non-cancer diseases. The assessment of chemical-induced mutagenicity is typically achieved using a battery of *in vitro* and *in vivo* tests. One limitation of the currently available, and widely used, *in vivo* genotoxicity tests, such as the *in vivo* chromosome aberration test and the micronucleus test, is that these methods assess genotoxicity by measuring the induction of chromosome damage rather than measuring mutagenicity directly. In contrast, transgenic rodent (TGR) gene mutation assays measure the ability of a chemical to induce mutations as the adverse effect. Since it is the potential of a chemical to induce mutations which is most often of interest, TGR assays can offer a significant advantage over many other genotoxicity tests.

Derek Nexus is an expert rule-based system for the prediction of toxicity. The knowledge base embedded in this software is composed of alerts, examples and reasoning rules which may each contribute to the toxicity predictions made by the system. Given the importance of assessing chemical-induced mutagenicity *in vivo*, structural alerts for this endpoint are clearly of value and TGR assays provide reliable data with which to derive these alerts. A recent collaborative project between the National Institute of Health Sciences of Japan (NIHS) and Lhasa Limited aimed to improve Derek Nexus alerts using a data set of TGR assays, provided by the NIHS, in order to modify or develop new alerts to further improve the coverage of the endpoint of *in vivo* mutagenicity in the Derek Nexus knowledge base. A data set of 188 unique compounds was compiled and provided to Lhasa Limited by the NIHS, summarising publicly available TGR assays data, in order to improve the prediction of such assays by Derek Nexus (Lambert et al., 2009). The TGR assay data provided by the NIHS can be associated with the endpoint of *in vivo* mutagenicity in Derek Nexus. The available TGR assays data were critically assessed by an expert at Lhasa in order to derive an overall result for each compound.

The project resulted in extension of twelve *in vitro* mutagenicity alerts and one chromosome damage alert to predict *in vivo* mutagenicity. Alerts covering a wide range of chemical space and mechanisms of mutagenicity were updated based on the newly available data. The strength of the data on which the scope of the alert was based was assessed based on both predictive performance and confidence in the expectation that the identified toxicophore is indeed responsible for the observed activity. A measure of confidence in alerts that are activated is reflected in the reasoning level provided by Derek Nexus, which allows the user to consider the likelihood of a potential hazard. The collaborative project has improved the coverage of the *in vivo* mutagenicity endpoint in Derek Nexus significantly: sensitivity rose from 10% to 57% while at the same time maintaining a good specificity of 89% for the current data set.

Reference

Lambert et al. (2009). Detailed review paper on transgenic rodent mutation assays. OECD Environment, Health and Safety Publications: Series on Testing and Assessment, No. 103, 1-128.

III-118

Evaluation of toxicological safety of antitumor prototypes with alternative methodology fish embryo test

Thallita Teixeira[1], Rebeca Goveia[1], Aliny Lima[1], Elisangela Silveir-Lacerda[1], Cesar Grisolia[2], Irvin Bryan Machado Ferraz[2], Rhaul de Oliveira[2] and Raquel Santos Faria[1]

[1]Instituto de Ciencias Biológicas, Universidade Federal de Goiás, Goiania, Brazil; [2]Instituto de Ciencias Biologicas, Universidade de Brasilia, Brasilia, Brazil

thallitam.ciencia@hotmail.com

Cancer is the main cause of death in the world. Antitumor drugs have toxic effects, so toxicity studies are necessary to development of a new drug. This study evaluates the toxicological safety of RuLAP(1), RuLAU(2), DPPE(3) and DPPM(4) prototypes by Fish Embryos Test. For the complex 1, hatching rate was observed above 50% in concentration 1.2 mg/L however, the complex 2, the hatch rate was less than 50% at all concentrations tested, for the 48 h period. In the 72 h and 96 h, the complex 1 and 2, showed a hatch rate above 40% at all concentrations tested and the mortality rate was higher than 50% only in 96 h period at concentrations 7.1 to 100 mg/L. For the complex 3 and 4, the hatching rate, in the periods 48 h, 72 h and 96 h, was greater than 30% in all concentrations tested. And the mortality rate was less than 20% in all concentrations tested (48 h to 96 h). Therefore, the results suggest that compounds 1 and 2 are more toxic, however, compounds 3 and 4 are less toxic to zebrafish embryos.

References

Araujo, G. L., Campos, M. A. A., Valente, M. A. S. et al. (2014). Alternative methods in toxicity testing: The current approach. *Braz J Pharm Sci 50*, 55-62.

Instituto Nacional do Cancer (INCA) (2016). Estimativa 2016: Incidencia do câncer no Brasil.

OECD (2013). OECD Guidelines for the testing of chemicals. Section 2: Effects on biotic systems test no. 236: Fish embryo acute toxicity (FET) test. Paris, France: OECD.

III-123

Kanagawa prefecture's approach for global standardization of Bhas 42 cell transformation assay: Non-genotoxic carcinogen-induced changes in gene expression over time on Bhas 42 cell transformation assay

Kiyomi Ohmori[1], Asuka Kamei[2], Yuki Watanabe[2] and Keiko Abe[2,3]

[1]Kanagawa Prefectural Institute of Public Health, Chigasaki, Kanagawa, Japan; [2]Kanagawa Institute of Industrial Science and Technology, Kawasaki, Kanagawa, Japan; [3]Graduate School of Agricultural and Life Science, University of Tokyo, Tokyo, Japan

ohkyon@33.catv-yokohama.ne.jp

As screening methods to predict carcinogenicity, genotoxicity assays have a major issue in that many carcinogens are negative in such assays. Non-genotoxic carcinogens, which thus do not induce DNA damage directly, are likely to be tumor-promoters. Therefore, Ohmori et al. (2004, 2005) developed "Bhas 42 cell transformation assay" to detect the focus formation activity of chemicals at the tumor-promotion stage. The assay was recognized by OECD as Guidance Document No.231 in 2016, it became the 1st internationally recognized *in vitro* tumor promotion assay. To provide evidence of the involvement of the chemical-induced focus formation of Bhas 42 cells in tumorigenesis and carcinogenesis, we conducted a transcriptome analysis over time by using DNA microarrays. The results suggested the mechanism underlying the focus formation in Bhas 42 cells and revealed the transformation-concurrent expression of various genes involved in cancers and tumors.

References

Ohmori, K., Sasaki, K., Asada, S. et al. (2004). An assay method for the prediction of tumor promoting potential of chemicals by the use of Bhas 42 cells. *Mutat Res 557*, 191-202.

Ohmori, K., Umeda, M., Tanaka, N. et al. (2005). An inter-laboratory collaborative study by the non-genotoxic carcinogen study group in Japan, on a cell transformation assay for tumour promoters using Bhas 42 cells. *Altern Lab Anim 33*, 619-639.

III-311

The HET-MN (Hen's Egg Test for Micronucleus-Induction): Promising pre-validation study

Bertrand Desprez[1], Kerstin Reisinger[2], Dagmar Fieblinger[3], Andreas Heppenheimer[4], Jochen Kuehnl[5], Juergen Kreutz[2], Manfred Liebsch[3], Andreas Luch[3], Katrin Maul[3], Albrecht Poth[4], Pamela Strauch[6], Wolfgang Voelkner[6], Thorsten Wolf[7] and Ralph Pirow[3]

[1]Cosmetics Europe, Brussels, Belgium; [2]Henkel AG & Co KGaA, Düsseldorf, Germany; [3]BfR, Berlin, Germany; [4]Harlan Cytotest Cell Research, Roßdorf, Germany; [5]Beiersdorf, Hamburg, Germany; [6]Envigo CRS GmbH, Roßdorf, Germany; [7]University of Osnabrück, Osnabrück, Germany

bdesprez@cosmeticseurope.eu

The HET-MN allows the analysis of micronuclei formation in erythrocytes of developing hen's eggs to address the systemic availability of compounds as it facilitates the absorption, distribution, and metabolism of compounds followed by their excretion into a bladder equivalent.

Here we present the results of 35 compounds, tested blinded in a pre-validation exercise in 3 laboratories covering true positives and negatives (concordant historical *in vitro* a. *in vivo* data) as well as misleading positives (positive *in vitro*, negative *in vivo*). Data analysis resulted in a specificity of 97% and a sensitivity of > 80%. Studies on xenobiotic metabolism proved the clear intrinsic metabolic capacity of the test system, which omits the need to add rat liver S9 mix.
According to our findings, the HET-MN is a promising assay to supplement existing *in vitro* genotoxicity test batteries to follow up on initial positive results.

The work was funded by the German Ministry for Res./Educ. and Cosmetics Europe.

III-508

Use of multiparametric *in vitro* mode of action approaches for genetic toxicity assessment

Stefan Pfuhler, Allemang Ashley, Nadira DeAbrew, Tom Downs and Yuching Shan

Procter & Gamble, Mason, OH, United States

pfuhler.s@pg.com

In support of the development of a predictive, animal-free genetic toxicology approach, we have investigated several different methods with varying degrees of complexity. Their predictive capacity was compared using 22 chemicals which were selected taking into consideration their modes of action, and expected *in vivo*/*vitro* genotoxicity outcomes. The simplest method, the ToxTracker reporter gene assay uses a panel of human stem cell lines, containing fluorescent reporter genes that represent DNA damage, oxidative stress, cellular stress, and protein damage. Two other methods involved the use of human lymphoblastoid TK6 cells and genomic analysis. For these studies, the chemicals were investigated in parallel by a flow cytometric micronucleus (MN) assay, cell viability estimates and were then analyzed using the Affymetrix Human Genome Array. Over-all predictivity of all methods was comparable (> 80%) and nearly all discrepancies between assays can be explained by dose selection.

III-643

High throughput, multiplexed assay for the characterization of DNA damage in early screening

Steven Bryce, Derek Bernacki, Jeff Bemis and Stephen Dertinger

Litron Laboratories, Rochester, NY, United States
sbryce@litronlabs.com

Our laboratory developed a multiplexed, add-and-read flow cytometric method – MultiFlow® – for the determination of genotoxic mode of action. In an effort to address early-screening environments and aid in the reduction and refinement of subsequent animal studies, we modified our approach by further miniaturizing the assay and studying a single time point. Exposure of TK6 cells to genotoxic and nongenotoxic agents (n = 24) was accomplished in 384 well plates. After 4 hours, MultiFlow reagent cocktail containing detergent, several Abs (γH2AX, phosphohistone H3, p53), DNA stain, and an absolute counting bead was added. Following 30 min incubation, analysis was accomplished by automated sample introduction which allowed walk-away operation. Initial examination of the data involved heat maps to examine response trends, followed by logistic regression modeling to categorize clastogens vs nongenotoxicants. Performance of the assay in correctly predicting *a priori* class was > 90%.

III-656

Integrating human cell panel based time-dependent cell response profile method coupled with micronucleus assay to detect and differentiate genotoxic chemicals

Can Jin, Diana Guimet and Yama Abassi

ACEA Biosciences, Inc., San Diego, CA, United States
cjin@aceabio.com

To assess genotoxic property of chemicals with pathway information on human health risks, a time-dependent cell response profile (TCRP) method was developed using three human cell lines. BEAS2B cells showed the most dramatic and consistent TCRP toward ~20 mutagens and clastogens, such as 5-fluorouracil and etoposide. HepG2 cell retained certain P450 activities which enabled detection of chemicals requiring metabolic activation, such as benzo(a)pyrene. Aneugens targeting microtubules induced a distinctive TCRP from clastogens in both cell lines. ACHN cells displayed a unique TCRP toward 30 HDAC inhibitors such as valproic acid. Sensitivity of the cell panel TCRP method to genotoxic chemicals is at least comparable to other sensitive cell based assays. In addition, the same HepG2 cells were processed at the end of TCRP assay for micronucleus (MN) analysis to differentiate clastogens from other mutagens, and to provide additional assessment on viability, cell cycle, and cytotoxicity. Altogether, the integrated TCRP and MN workflow is the first *in vitro* method we know that can detect and differentiate multiple subgroups of genotoxic compounds.

References

Atienza, J. M. et al. (2005). Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays. *J Biomol Screen 10*, 795-805.

National Research Council (2007). *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Washington, DC: The National Academies Press.

OECD. (2014). Test No. 487. Guideline for the Testing of Chemicals: In Vitro Mammalian Cell Micronucleus Test.

Yamashita, Y., Shimada, M., Harimoto, N. et al. (2013). Histone deacetylase inhibitor trichostatin A induces cell-cycle arrest/apoptosis and hepatocyte differentiation in human hepatoma *Cells*. *Int J Cancer 103*, 572-576.

Yun, C.-H., Shimada, T. and Guengerich, F. P. (1992). Roles of human liver cytochrome P4505C and 3A enzymes in the 3-hydroxylation of benzo(a)pyrene. *Cancer Res 52*, 1868-1874 (April 1, 1992)

III-741

AMES/QSAR international collaborative study

Masamitsu Honma

National Institute of Health Sciences, Tokyo, Japan
honma@nihs.go.jp

Robust *in silico* toxicity prediction enables identification of chemicals with potential adverse effects while reducing the need for experimental studies. Accurately estimating the likelihood of being genotoxic is crucial in regulatory programs including ICH M7. Quantitative Structure-Activity Relationships (QSAR) and expert knowledge-based rules are commonly employed for *in silico* models, and are well-suited for DNA-reactive mutagenicity where modes of chemical reactivity are well characterized. In 2014, the Division of Genetics and Mutagenesis (DGM) at the National Institute of Health Science (NIHS) in Japan launched the Ames/QSAR international collaboration with regulatory Ames mutagenicity data for over 12,000 new chemicals. Three phases were designed to allow testing of the hypothesis that the knowledgebase expansion enhances prediction results. Each phase provides about 4000 chemicals with three classifications: Class A (strong positive, 5-6%), Class B (positive, 8-10%), and Class C (negative, 85%). Among the 12 QSAR models, sensitivities ranged 39-70% (Phase I) and 42-68% (Phase II); specificity values ranged 65-92% (Phase I) and 78-93% (Phase II). The improved performance was indeed observed after modelers incorporated test compounds from phase I into their training sets for phase II.

Innovative Models for Safety and Efficacy – Regulatory Issues Related to Organs on a Chip

III-148

Improved IC_{50} prediction using the Quasi Vivo® *in vitro* dynamic cell culture flow system

Kelly Davidge and J. Malcolm Wilkinson
Kirkstall Ltd, Rotherham, United Kingdom
kelly.davidge@kirkstall.com

There is currently great interest in applying microfluidics technology to the construction of complex *in vitro* models with the eventual goal of simulating the behaviour of an organ or even a whole organism. The enthusiasm for microtechnology has been generated by past success in scaling cell culture from a petri dish down to the current multiple well plates. However, our gold standard for clinical response is the human body, which incorporates functional units that require larger scale. It is widely recognised that 3D cell cultures are more representative of human physiology than 2D monolayers of cells growing on a flat surface. Correct organ function also requires gradients of oxygen and metabolites and removal of waste material, as well as co-culture of multiple cell types – hence advanced and accurate models also require flow of media.

The goal for organ-on-a-chip is to accurately mimic clinical behaviour. Primary cells can be provided with a more physiologically-relevant environment via the introduction of dynamic flow during cell culture. Primary hepatocytes cultured in a collagen sandwich show more human-like expression of key detox genes (Vinci et al., 2011) and fibroblasts cultured under flow show vastly different gene expression profiles compare with those cultured in static conditions (Nithiananthan et al., 2016).

Using primary hepatocytes, we show that flow conditions can significantly upregulate phase I and phase II metabolic enzyme genes and this is reflected in the metabolism of certain tested drugs. In line with the mRNA data, CYP2D6 (dextromethorphan) was not influenced by flow, but both phase I and II metabolism of Midazolam (CYP3A4 and UGT) were strongly upregulated. When challenged by diclofenac for up to 24 hours, primary rat hepatocytes give a more realistic assessment of toxicity than classical methods, predicting an IC_{50} of around 50 μM compared with around 500 μM in static conditions; toxicity has been seen in the clinic at 4.2 μM. Diclofenac causes a detectable 10% toxicity in the Quasi Vivo® at this concentration but toxicity cannot be distinguished in static cultures. We have also shown that the Quasi Vivo® system is 50% more accurate in predicting the IC_{50} for APAP and cyclophosphamide-primary rat hepatocytes cultured under flow are more sensitive to these drugs than those cultured under static conditions.

In summary, using the Quasi Vivo® technology, cells cultured under optimum flow conditions are more metabolically competent than those cultured using traditional static techniques, and it appears that cells in interconnected chambers can create homeostatic conditions; this is achieved through the maintenance of oxygen and nutrient conditions and less frequent medium changes leading to a more stable culture with less human interaction. Results obtained using the Quasi Vivo® system are more human-relevant than both traditional *in vitro* technique and *in vivo* studies; as such, it is an ideal *in vitro* tool for animal replacement.

References

Nithiananthan, S., Crawford, A., Knock, J. C. et al. (2016). Physiological fluid flow moderates fibroblast responses to TGF-β1. *J Cell Biochem 13,* 1-13.

Vinci, B., Duret, C., Klieber, S. et al. (2011). Modular bioreactor for primary human hepatocyte culture: Medium flow stimulates expression and activity of detoxification genes. *Biotech J 6*, 554-564.

III-217

Integrating human immuno competence into a Multi-Organ-Chip platform

Juliane Hübner[1], Ulrike Schließer[2], Jana Reuschel[2], Christoph Giese[2] and Uwe Marx[1]
[1]TissUse GmbH, Berlin, Germany; [2]ProBioGen AG, Berlin, Germany
juliane.huebner@tissuse.com

Microphysiological systems enabling long-term co-culture of various human organ equivalents have become increasingly useful for preclinical systemic drug testing. Until now, human lymphatic cells and lymphoid tissue are not integrated into these systems and therefore don´t generate human immune responses or have any regulative immune homeostasis. This hamper their use for the evaluation of immunotoxic or immunological active substances, e.g. vaccines. Here we report the generation and long-term cultivation of human lymphoid tissues in the micro-organoid format in a commercial multi-organ-chip platform. Therefore, peripheral blood mononuclear cells (PBMCs) were inoculated into a multi-organ-chip compliant hydrogel matrix. Robust culture procedures were established to keep the lymphoid organoids viable and functionally responsive. Comparative data on cell composition, migration and cluster formation within the lymphoid tissues with and without pre-stimulation of the PBMCs are presented.

III-263

Investigating the effects of nicotine using a human Multi-Organ-Chip approach

Isabel Rütschle[1], Stefan Frentzel[2], Karsta Luettich[2], Arno Knorr[2], Katrin Brandmair[1], Alexandra Lorenz[1], Ilka Maschmeyer[1], Juliane Hübner[1], Uwe Marx[1] and Julia Hoeng[2]

[1]TissUse GmbH, Berlin, Germany; [2]Philip Morris Products S.A., Neuchâtel, Switzerland

isabel.ruetschle@tissuse.com

TissUse's Multi-Organ-Chip (MOC) platform provides preclinical insight on a systemic level using human tissue and enables the prediction of effects of chemicals and their metabolism on near real-life models. In order to be able to elucidate the toxicity of inhaled compounds, we adjusted the MOC design for the optimal co-cultivation of a human liver equivalent and the MucilAir™ lung model. Tissue integrity and function were evaluated using metabolic analysis, measurement of trans-epithelial electrical resistance and cilia beat frequency, immunohistochemistry, gene expression analysis and albumin quantification. Viability and homeostasis could be demonstrated for 14-day lung/liver co-cultures. Initial tests with nicotine, applied either apically to the lung model or systemically, indicated active compound metabolism and the utility of the current MOC set-up for future research into the biological effects of inhaled compounds on the homeostatic co-culture.

Innovative Models for Safety and Efficacy – 3D Organ Models

III-125

RAFT™ 3D cell culture system provides a versatile platform for co-culture and barrier models

Theresa D'Souza, John Langer and Therese Willstaedt
Cell Biology R&T, Lonza Walkersville Inc., Walkersville, MD, United States
theresa.dsouza@lonza.com

Skin and lung tissues are some of the specialized barrier tissues used in *in vitro* test systems in predicting and providing dependable pre-clinical research data. Three-dimensional (3D) cell culture systems aim to provide cells from these barrier tissues with a more natural growth environment with the help of e.g. hydrogel matrixes or synthetic scaffolds. Collagen, in particular collagen type I, is one of the most abundant extracellular matrix proteins in the body and therefore an often-used 3D cell culture material. Lonza offers the novel RAFT™ (Real Architecture for Tissue) 3D Cell Culture system that allows the creation of tissue-like structures with cells growing within or atop a high-density collagen scaffold. We present data here demonstrating differences in a lung co-culture model in a two-dimensional (2D) mode and in the RAFT™ 3D cell culture system using Clonetics™ normal and asthmatic bronchial epithelial cells and smooth muscle cells. The cell proliferation, morphology, growth factors and cytokines were investigated for both 2D and 3D systems. We also demonstrate full-thickness skin using Clonetics™ keratinocytes and fibroblasts in the RAFT™ 3D cell culture system. Evaluation by histology and immunofluorescence markers confirmed the resemblance to native skin. These 3D cell culture systems provide a valuable tool to investigate barrier tissues in an *in vivo*-like microenvironment, potentially for use in pre-clinical efficacy and safety testing.

III-167

Maintenance of viability and functional expression of cryopreserved human hepatocytes using silicate fiber-based three-dimensional scaffold

Taku Matsushita[1], Takahiro Mizutami[1], Hanaka Nagasaki[1], Takaaki Ishii[1], Yuji Komizu[1], Takuya Iwasa[2], Masaaki Kawabe[2] and Seiichi Ishida[3]
[1]Department of Applied Life Science, Sojo University, Kumamoto, Japan; [2]Central Research Laboratory, Japan Vilene Co., Ltd, Ibaraki, Japan; [3]Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan
matusita@life.sojo-u.ac.jp

The development of a cell culture maintenance system is important for alternatives to animal testing and experimentation for drug discovery. Generally, cryopreserved human hepatocyte viability and function are maintained for about 3 days after thawing in 2D monolayer culture using a collagen-coated dish. In this study, we cultured human hepatocytes in the silicate fiber-based 3D scaffold Cellbed. As a result of observation with SEM, hepatocytes were cuboidal as compared with 2D culture, which seems to be appropriate for maintenance of cell viability and function of hepatocytes. Cell viability in 3D culture was maintained at a higher level and for about 2 weeks than that of 2D culture. Levels of GOT and GPT leakage were also lower in 3D culture than in 2D culture. Based on gene expression analysis, *CYP2C19* and *CYP3A4* were expressed in 3D culture at levels more than 3.0 times higher than those of 2D culture. Moreover, *SLC22A1*, the gene for bile acid transporter, was expressed in 3D Cellbed culture at a level more than 2.9 times higher than that in monolayer. This culture system may be applied to hepatic metabolism studies and long-term *in vitro* liver toxicity testing.

III-221

Modulation of hepatic progenitor and stellate cell fate by VECELL 3-D insert

Seiichi Ishida[1], Shinichiro Horiuch[1], Mitsuko Hara[2], Yukie Kuroda[1], Shoko Uchida[1], Su-Ryang Kim[1], Tomokazu Matsuura[3], Fabrice Morel[4], Anne Corlu[4] and Soichi Kojima[2]
[1]National Institute of Health Sciences, Tokyo, Japan; [2]RIKEN, Wako, Japan; [3]The Jikei University, Minato, Tokyo, Japan; [4]INSERM, Strasbourg, France
ishida@nihs.go.jp

VECELL 3-D insert (VECELL) is a culture vessel scaffold consisting of an expanded polytetrafluoroethylene (ePTFE) mesh coated with salmon collagen. VECELL is able to put cells into environment close to *in vivo* situation and maintain them in native round shape because there is no excess room for cells to stretch. Therefore, it is expected that cells cultured on VECELL show cell state different from that cultured on standard culture plate. We have applied VECELL to various hepatic cell cultures and found that it maintains cells in immature state. Hepatic progenitor cell line HepaRG cultured on VECELL maintained round shape and undifferentiated state for a long period of cultivation. When activated hepatic stellate cells were cultured on VECELL, they formed spheroids and were deactivated. Thus, VECELL was able to modulate the cell fate, and might be a unique culture apparatus to provide different kinds of cell sources.

Reference

Kubo, T., Kuroda, Y., Hojyo, M. et al. (2016). Maintenance of hepatic progenitor-like characteristics of HepaRG cells by cultivation on VECELL Inserts. *AATEX 21*, 62-69.

III-230

GravityFLOW: A microtissue-based multi-tissue platform

Lei He[1], Christian Lohasz[2], Kasper Renggli[2], David Fluri[3], Andreas Hierlemann[2] and Olivier Frey[3]

[1]InSphero Inc, Brunswick, ME, United States; [2]ETH Zurich, Basel, Switzerland; [3]InSphero AG, Schlieren, Switzerland

lei.he@insphero.com

Multi-tissue setups or so-called microphysiological systems (MPS) interconnect several organ models through microfluidic technology and have the potential to investigate the action of compounds in a more systemic, *in vivo*-like fashion. In the pre-clinical drug development stage, such insights are currently only available using animal experiments.

The increased biological and technical complexity of MPS poses challenges on liquid and tissue handling as well as experimental reproducibility. We address them with a simple and robust 96-well format-based microfluidic platform that enables multi-tissue networks using microtissue spheroids. Multiple devices, each including 8 separate channels and each channel containing 10 compartments for 10 same or different interconnected microtissues can be operated in parallel.

The miniaturized format substantially reduces cell and medium consumption, especially from animal origin. Further, we focus on human cells. Continuous perfusion and increased cell-to-liquid ratios significantly improves microtissue functionality and better reproduces *in vivo*-like behavior.

III-243

Use of newly validated 3D reconstructed human skin genotoxicity assays and hens egg test micronucleus in testing strategies improve predictions in safety assessments of cosmetic ingredients

Rolf Fautz[1], Bertrand Desprez[2], Sebastian Hoffmann[3], Nicky Hewitt[4], Paul Fowler[5], Kerstin Reisinger[6], Jochen Kuehnl[7], Gladys Ouedraogo[8], Julia Kenny[9] and Stefan Pfuhler[10]

[1]Kao Germany GmbH, Darmstadt, Germany; [2]Cosmetics Europe, Brussels, Belgium; [3]seh consulting + services, Paderborn, Germany; [4]Nicky Hewitt Scientific Writing Services, Erzhausen, Germany; [5]Unilever, Sharnbrook, Bedford, United Kingdom; [6]Henkel AG & Co. KGaA, Duesseldorf, Germany; [7]Beiersdorf AG, Hamburg, Germany; [8]L'Oréal Life Sciences Research, Aulnay sous Bois, France; [9]GSK, Ware, United Kingdom; [10]Procter & Gamble, Mason, Cincinnati, OH, United States

rolf.fautz@kao.com

The reconstructed skin micronucleus (RSMN) and the 3D skin Comet are genotoxicity tests employing reconstructed human skin tissues. They have been recently validated and showed very promising results, with good sensitivity, specificity. The hen's egg micronucleus test (HETMN) also had a very good predictive capacity in recent validation studies. These tests are relevant for the safety assessment of cosmetics because they incorporate metabolism and exposure scenarios (topical or oral). Their high predictive capacities, and especially their excellent specificities, makes them ideal as follow up tests to the current battery 2-test battery (Ames and *in vitro* micronucleus tests) recommended for the genotoxic assessment of cosmetics, which tend to result in misleading positive results. We report here the overall performance of these three assays and their use in a safety assessment strategy.

III-259

Genetic engineering of a 3D *in vitro* human lung cancer model

Samuel Constant[1,2], Hugo Benainous[2], Song Huang[1], Ludovic Wiszniewski[1] and Christophe Mas[2]

[1]Epithelix, Plan-les-Ouates, Switzerland; [2]OncoTheis, Plan-les-Ouates, Switzerland

samuel.constant@epithelix.com

Lung cancer is a top killer among cancers with a mortality rate up to 70% within 1 year after diagnosis. Clearly, realistic human 3D models recapitulating interactions between tumour and its original microenvironment are required to improve preclinical predictivity. Here, by engineering oncogenic KRAS and TP53 mutations into an air-liquid reconstituted airway, we developed a fully humanized lung cancer model which replicates the progression of the disease. Remarkably, oncogene transformed tissue areas showed significant dysplasia with marked areas of invasion. Enlarged regions displayed increased KI67 proliferation rate and high P63 expression. Of note, KRAS-P53 double mutants showed a stronger phenotype, with higher dysplasia index, compared to the single mutants. Our results underline the potential of this early stage committed cancer model to study *in vitro* the molecular mechanisms involved in lung carcinogenesis but also the effect of carcinogens exposure on lung cancer progression.

III-622

Non-invasive, real time monitoring of cellular function and assessment of chronic toxicity in 4-organ microfluidic platform

Carlos A. Carmona-Moran[1], Andrea Caetano Das Neves Lavado[1], Carlota Oleaga-Sancho[1], Sandra Rothemund[1], Yunquing Cai[1], Case Lorance[1], Richard L. Bridges[1], Mark Schnepper[1], Christopher McAleer[1], Christopher J. Long[1], Jessica Langer[2], Anne Riu[3], Reine Note[3], Silvia Teissier[3], Jose Cotovio[3], Michael L. Shuler[4] and James J. Hickman[1]

[1]NanoScience Technology Center, University of Central Florida, Orlando, FL, United States; [2]L'Oréal, Research and Innovation, Clark, NJ, United States; [3]L'Oréal, Research and Innovation, Aulnay sous Bois, France; [4]Department of Biomedical Engineering, Cornell University, Ithaca, NY, United States

carlosar@ucf.edu

Systemic toxicity for cosmetics is challenging due to animal bans and insufficient *in vitro* models, leading to the development of alternative human tissue models. "Body-on-a-chip" systems use functional cell assays reflecting multiple organs, with recirculating medium scaled to physiologic levels, to evaluate potential toxicity. We show a human 4-organ chip under constant, pump-less flow in a serum-free, defined medium for 28 days. Functions were assessed by analyzing electric and mechanical cardiac function, neuronal activity, muscle contraction, hepatic enzyme, albumin and urea profiles, combined with a broad metabolic panel. The system allows non-invasive real-time readouts for acute and chronic measurements. 28-day viability was demonstrated as well as functional cardiac, skeletal muscle, neuronal and liver modules responses to different drugs, compared to clinical data. This is a novel *in vitro* model for repeated dose toxicity prediction.

Innovative Models for Safety and Efficacy - Exposure Models

III-66

H_2O_2 as an oxidative stressor in a degeneration model of a pig retina organ culture

Sandra Kuehn[1], José Hurst[2], Adelina Jashari[1], Teresa Tsai[1], H. Burkhard Dick[1], Sven Schnichels[2] and Stephanie Joachim[1]

[1]University Eye Hospital, Ruhr-University, Bochum, Germany; [2]University Eye Hospital, Tübingen, Germany
sandra.kuehn@rub.de

Purpose: We want to establish an *ex-vivo* model with pig retinas from the abattoir for eye diseases in which oxidative stress is a key player to reduce the animal number in retinal research.

Methods: Organotypic pig retina explants were cultivated and treated with different doses of hydroxyl peroxide (H_2O_2; 100, 300, 500 μM) for 3 h on day 1. At day 3 and 8, retinas were analyzed.

Results: At day 3, the expression of *INOS* was increased ($p < 0.05$). At day 8, apoptotic ($p < 0.05$) loss of retinal ganglion cells (RGCs) was noted ($p < 0.05$). Furthermore, the activation state of the microglia was increased ($p < 0.05$) and also the expression of TNFα ($p < 0.05$).

Conclusions: H_2O_2 turns on oxidative stress cascades, which results in an apoptotic loss of RGCs accompanied by a microglia response. Therefore, a standardized organ culture model for oxidative stress was successfully established as a new prescreening model of potential ophthalmic therapies without the need to euthanize any additional animals.

III-73

Mimicry of hypoxic mechanism induced by cobald-chloride in a novel porcine retina organ culture model

Teresa Tsai[1], Sandra Kuehn[1], José Hurst[2], Farina Rensinghoff[1], Sven Schnichels[2] and Stephanie C. Joachim[1]

[1]Experimental Eye Research, University Eye Hospital, Ruhr-University Bochum, Bochum, Germany; [2]University Eye Hospital Tübingen, Centre for Ophthalmology Tübingen, Tübingen, Germany
teresa.tsai@rub.de

Purpose: Cobalt is important for the neuronal integrity; however high quantities induce cytotoxic mechanisms. We tested the degenerative effect of cobalt-chloride ($CoCl_2$) in a porcine retina organ culture, to establish an alternative retina degeneration model.

Methods: Organotypic cultures of porcine retinae were cultivated and treated with different concentrations of CoCl2 (100, 300 and 500 μM) for 48 h.

Results: At day 8, 300 and 500 μM $CoCl_2$ reduced the retinal ganglion cell (300 μM: $p = 0.002$; 500 μM: $p < 0.001$), amacrine cell (300 μM: $p = 0.002$; 500 μM: $p = 0.001$) and bipolar cell number (300 μM: $p = 0.007$; 500 μM: $p = 0.001$). In addition, all three $CoCl_2$ concentrations reduced the microglia population ($p < 0.05$).

Conclusions: $CoCl_2$ induced a strong degeneration of the inner retina layers and microglia, starting at 300 μM. A promising alternative model for hypoxia induced retinal degeneration could be established, which uses pig eyes from the abattoir and is suitable for therapy screening.

III-137

Getting more from your dose response curves

James Miller, Jia-Ying Joey Lee and Lit-Hsin Loo

Bioinformatics Institute, A*STAR, Singapore
jamesam@bii.a-star.edu.sg

Dose response curves (DRCs) are widely used for assessing chemical toxicity. DRC metrics used in *in vitro* toxicity assays usually have the dimensions of concentration, such as "half-maximal activity concentration" (AC_{50}) or "lethal concentration to n% of a cellular population" (LCn). The advent of high-content screening has enabled the automated assessment of complex cellular phenotypic responses (e.g. cell morphology or protein localization). What are the optimal DRC metrics for toxicity prediction with these responses?

We present a performance study of different DRC metrics, classifying three high-content screening datasets for nephro- and pulmonotoxicity. DRC metrics which measure phenotypic responses tend to be more predictive than metrics which measure concentrations. Therefore, toxicity may be more correlated with a chemical's efficacy than with its potency. This has implications for the design of toxicity assays, and the selection of the most predictive test concentrations.

III-171

Acute oral toxicity modeling accounting for mechanism and toxicological mode of action

Ann Detroyer[1], D. Nedelcheva[2], R. Note[1], N. Dimitrova[2], D. Blanchet[1], S. Stoeva[2], A. Fadli[1], K. Kirilov[2], S. Dimitrov[2], S. Ringeissen[1] and Ovanes Mekenyan[2]

[1]L'Oréal R&I, Aulnay sous Bois, France; [2]Laboratory of Mathematical Chemistry, University "Prof. As. Zlatarov", Bourgas, Bulgaria
adetroyer@rd.loreal.com

This work aimed at developing an *in silico* modelling system meeting the alternative method's requirements for acute oral toxicity (AOT) of chemicals previously reported from assessment on rats. Thus, the system is especially designed to provide a mechanistic justification and transparency of the predictions.

The model development is based on a search in the experimental database of known rat LD_{50} data, next on categorizing chemicals based on their functionality, potential mechanism of interaction with cell compartments and biological effect. For each category of chemicals, if possible, a local QSAR model is developed accounting for the bioavailability of chemicals and their pattern of toxicity.

Currently, the AOT modeling system implemented in the TIMES platform includes 2694 training set compounds, comprising 84 toxicological categories. All predictions are to be supported by mechanistic justification for the mode of action, example chemicals and an indication of applicability domain.

III-196

HSE management for a Ti-based nano-additive material: From core-shell production to final part

Sandra Verstraelen, Lieve Geerts, Reinhilde Weltens, Karen Hollanders, An Jacobs, Guy Geukens, Jo Van Laer, Inge Nelissen and Evelien Frijns

Flemish Institute for Technological Research, Research Group for Environmental Risk and Health, Mol, Belgium
sandra.verstraelen@vito.be

In the nanosafety arena huge efforts are being made to develop the Safe-by-Design concept as a way to incorporate the Health, Safety, and Environmental (HSE) aspects in an early stage of the innovation process in order to guarantee safety at the workplace, for consumers, and the environment.

VITO started to implement this concept in the NANOTUN3D project concerning the development of nano-modified alloys for additive manufacturing. During nanoproduct development, exposure scenarios at each life cycle stage are identified. Along the life cycle, on-site exposure measurements are performed to determine and confirm hot spots of human exposure, and to indicate preventive measures. Impact on human health hazard is assessed by *in vitro* studies of nanomaterial interactions with human cells, and also ecological hazard is identified. These data, together with available data from literature and physico-chemical characteristics is integrated to obtain a HSE management system.

III-661

Development of a novel computational workflow to predict nicotinic acetylcholine (nAChR) receptor-mediated acute mammalian intravenous toxicity

Sanjeeva Wijeyesakere, Daniel Wilson, Tyler Auernhammer, Amanda Parks and Sue Marty

Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, MI, United States
SWijeyesakere@dow.com

Given recent trends towards reducing laboratory animal use, we developed a computational model to predict the acute toxicity of chemicals that interact with the nAChR, a major target for pharmaceuticals and an off-target of toxicity for industrial chemicals. By curating a database of over 3400 nAChR agents followed by molecular scaffolding, we identified 545 scaffolds that describe the chemical space of nearly all known nAChR agents with high sensitivity (94.8%) and specificity (60%). Also, using a regional molecular docking algorithm, we constructed a set of regression models to predict intravenous LD_{50}s for several classes of nAChR agents with high precision ($r^2 > 0.7$). Thus, we highlight the utility of a computerized workflow to identify key scaffolds in nAChR agents and the structural basis for their interaction with the nAChR. We expect these findings to be broadly applicable towards building models to screen novel chemicals for interaction with other cellular targets of toxicity.

Innovative Models for Safety and Efficacy – Innovations in Drug Development

III-15

Alternatives and animal use in medicinal mushroom research

Humberto Morris[1], Gabriel Llaurado[1], Yaixa Beltran[1], Lisandra Cutiño[1], Javier Peña[1], Pedro Luis Batista[2], Serge Moukha[3,4], Paul Cos[5], Isabelle Gaime-Perraud[6], Rosa Catalina Bermudez[1] and Nora Garcia[1]

[1]Center for Studies on Industrial Biotechnology (CEBI), Universidad de Oriente, Santiago de Cuba, Cuba; [2]Center of Toxicology and Biomedicine, Medical University of Santiago de Cuba, Santiago de Cuba, Cuba; [3]Department of Toxicology and Applied Hygiene, UFR des Sciences Pharmaceutiques-Université Bordeaux Segalen, Bordeaux, France; [4]INRA, Mycologie et Sécurité des Aliments, Villenave d'Ornon, France; [5]Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium; [6]Institut Mediterranéen de Biodiversité et d'Écologie (IMBE), Marseille, France
jquevedo@uo.edu.cu

The 3Rs (reduce, replace and refine) serve as guiding principles for humane animal-based research. Pharmaceutical companies involved in drug discovery need new sources of natural products and in a short amount of time, medicinal mushrooms can be used in the production of new pharmaceuticals. With the recent advancements in medicinal mushroom science, it is an opportune time to revisit and reconsider how these guiding principles may be applied to the drug discovery pathway for mushroom bioactive metabolites. This work will introduce some basic steps for an integrated research platform covering the *in silico*, *in vitro* and *in vivo* animal models prior to clinical drug development. *In silico* studies allow the prediction of antitumor activity of low molecular weight compounds (e.g. mushroom phenolics such as flavonoids) and their putative mechanisms of action (MOAs) based on chemical and structural similarity patterns. Moreover, with this *in silico* approach, the modulation of epigenetic events (e.g. histones acetylation/deacetylation and DNA methylation) could be investigated. Depending on the main target of the study, a specific cell line (human and/or animal) should be selected for *in vitro* studies. We tested the effect of several extracts of obtained from fruiting bodies of a *Pleurotus* sp. (oyster mushroom, Basidiomycetes) on growth of different cell lines (NB4, U937, HepG2, N2A and Caco2 tumor cells compared to Vero cells). Inhibition of cell proliferation, using the MTT and neutral red methods, was observed with low temperature aqueous extracts, while hot water and methanolic extracts were less efficient. *In vitro* antioxidant potential was also demonstrated by means of radical scavenging tests, reducing power and inhibition of lipid peroxidation in erythrocytes membrane. Extracts or fractions that showed a desired effect on *in vitro* assays were tested for their *in vivo* effect in animal models. Developing effective validated animal models is recognized as a sensitive issue for obtaining consistent results in the evaluation of medicinal mushrooms products in the quest for treatments and cures. *Pleurotus*-derived preparations exerted a stimulant effect on hemopoiesis and cell immune response in both immune competent and immunodeficient (cyclophosphamide-treated and whole-body irradiated) BALB/c mice. It was evident by increases in bone marrow cellularity, leukocyte counts, the stimulation of macrophage phagocytic activity and the reconstitution of delayed-type hypersensitivity reaction (DTH). These animal studies reflected that attempts to "domesticate" the immune system for the benefit of man, in addition to specific vaccines and antibodies, would find in mushrooms new and unlimited possibilities of exogenous molecules. As a result, our findings provided the basis for submitting to the national regulatory agency, the first Cuban dietetic supplement designed from mushroom material "NUTRISETAS®", a candidate with potential applications for immunonutrition and immunotherapy practices. More studies are needed to demonstrate which mushroom extracts or compounds are more effective for specific ailments (cancer, immunosuppression, metabolic syndrome, viral and bacterial infections, etc.). To accomplish these goals in present and future investigations, the role of the classic 3Rs of humane animal science as well as a new R, "reproducibility", should be encouraged together with the continue education and training of mushroom scientists in these issues. The rationale combination of alternatives and animal use in the illustrated *in silico-in vitro-in vivo* platform offers perspectives when addressing efforts for ethics and lab animals' welfare.

III-107

Preclinical assessment of seizure liability of drugs *in vitro*

Mohamed Kreir[1], Bruno Van Deuren[1], Sofie Versweyveld[2], Henk Van der Linde[1], Lu Hua Rong[1], Greet Teuns[1] and David Gallacher[1]

[1]Global Safety Pharmacology, Preclinical Development and Safety, Discovery Sciences, Janssen Pharmaceutica NV, Beerse, Belgium; [2]Neuroscience Discovery, Janssen Research & Development, Janssen Pharmaceutica NV, Beerse, Belgium
mkreir@its.jnj.com

Drug-induced seizures contribute to the high attrition rate of pharmaceutical compounds in development and the assessment of drug-induced seizures usually occurs in the later phases of the drug discovery process using low throughput and intensive *in vivo* assays. We are evaluating the potential of an *in vitro* assay for detecting drug-induced seizures. We investigated 10 reference drugs, of known seizurogenic risk using multi-electrode arrays (MEA) recordings of freshly-dissociated rat primary neurons and compared the results to those obtained in anesthetized rats. Our data show that the *in vitro* assay has advantages over the *in vivo* rat model in terms of throughput with lower compound requirements, higher sensitivity and more effective prediction of seizure risk. Moreover, we are currently in the early stages of comparing rat primary neurons versus human iPSCs. As such we have the potential to reduce and refine animal use (2Rs) within our Drug Discovery and Development process.

Innovative Models for Safety and Efficacy – Intestinal

III-150

Cytotoxicity and permeability of papain-cyclodextrin complex across Caco-2 cell monolayers

Fúlvio Corazza, Felipe Nambu, Vânia Leite-Silva, Newton Andréo-Filho and Patricia Lopes

Ciências Farmacêuticas, Universidade Federal de Sao Paulo, Diadema, Brazil

fcfulviocorazza@gmail.com

Papain is a proteolytic enzyme used to promote treatment and healing of wounded tissues and also as an agent to increase oral permeability. The aim of this study was to evaluate the cell viability and the permeability of papain-cyclodextrins complex in a Caco-2 cell model. The biophysical cell monolayer integrity was evaluated by TEER. The P-gp efflux was estimated by fluorescence measuring (apical and basolateral direction) using rhodamine 123 and verapamil hydrochloride. The paracellular permeability was assayed by fluorescence measuring of Lucifer yellow (apical to basolateral transport compartment) to assess the cell monolayer integrity during the experiment. Papain complex showed low cytotoxicity and high relative permeability due to its trypsin-like activity and TEER was strongly affected. In conclusion papain complex could be used in oral drug delivery systems (colonic permeability enhancer) due to its therapeutic properties, low cytotoxicity and elevated *in vitro* permeability.

References

Antoine, D., Pellequer, Y., Tempesta, C. et al. (2015). Biorelevant media resistant co-culture model mimicking permeability of human intestine. *Int J Pharm 481*, 27-36.

Béduneau, A., Tempesta, C., Fimbel, S. et al. (2014). A tunable Caco-2/HT29-MTX co-culture model mimicking variable permeabilities of the human intestine obtained by an original seeding procedure. *Eur J Pharm Biopharm 87*, 290-298.

Hubatsch, I., Ragnarsson, E. G. and Artursson, P. (2007). Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nat Protoc 2,* 2111-2119.

III-199

Toxic effects of beauvericin on an *in vitro* model of intestinal barrier

Francesca Caloni[1], Marco Albonico[1], Luca Sinisi[1], Virginia Bassi[1], Paola Pocar[1] and Cristina Cortinovis[2]

[1]Università degli Studi di Milano Dipartimento di Medicina Veterinaria (DIMEVET), Milan, Italy; [2]Università degli Studi di Milano Department of Health, Animal Science and Food Safety (VESPA), Milan, Italy

francesca.caloni@unimi.it

Beauvericin (BEA) is a mycotoxin synthesized by several *Fusarium* spp. parasitic to important cereal grains. To evaluate the effects of BEA on the intestinal barrier Caco-2 cells were cultured on semi-permeable inserts and, after differentiation, exposed for 24 h to BEA (0, 0.5, 1.5, 3, 6 μM) from the apical (Ap) or basolateral (Bl) side. BEA effects on the trans-epithelial electrical resistance (TEER) and interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) release were determined. TEER was not significantly affected by Ap exposure to all doses of BEA. On the contrary, a significant ($P < 0.05$) TEER decrease was observed after Bl exposure to BEA at 0.5 and 1.5 μM starting from the first hour of exposure. At 3 and 6 μM, BEA significantly ($P < 0.05$) increased TEER after 24 h of Bl exposure. A significant release ($P < 0.05$) of the inflammatory mediators IL-6 and IL-8 was induced by Ap and Bl exposure to BEA at 3 and 6 μM. No significant release of TNF-α was observed.

Reference

Jestoi, M. (2008). Emerging fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: A review. *Crit Rev Food Sci Nutr 48*, 21-49.

III-258

Toxicity of fumonisin B1 on the human intestinal cell line Caco-2

Francesca Caloni[1], Marco Albonico[1], Luca Sinisi[1], Virginia Bassi[1], Paola Pocar[1] and Cristina Cortinovis[2]

[1]Dipartimento di Medicina Veterinaria (DIMEVET), Università degli Studi di Milano, Milan, Italy; [2] Department of Health, Animal Science and Food Safety (VESPA), Università degli Studi di Milano, Milan, Italy

francesca.caloni@unimi.it

Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium* species frequently occurring in corn and corn by-products worldwide. This study aimed to evaluate the toxic effects of FB_1 on human intestinal Caco-2 cells cultured on semi-permeable inserts. After differentiation, Caco-2 cells were treated for 24 h with FB_1 (0, 0.5, 1.5, 3 μM) from the apical (Ap) or basolateral (B_1) side. Barrier impairment was assessed by measuring the trans-epithelial electrical resistance (TEER) after 1 h, 2 h and 24 h of treatment. At the end of the treatment, medium was collected for interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) determination. No significant TEER variations were observed after 24 h of Ap or B_1 exposure to all doses of FB_1. At the highest concentration tested (3 μM), FB_1 was found to induce a significant ($P < 0.05$) release of the pro-inflammatory cytokine IL-8. No significant release of IL-6 and TNF-α was observed after Ap or Bl exposure to FB_1 at all doses.

Reference

Minervini, F., Garbetta, A., D'Antuono, I. et al. (2014). Toxic mechanisms induced by fumonisin b1 mycotoxin on human intestinal cell line. *Arch Environ Contam Toxicol 67*, 115-123.

III-337

Development of a predictive screening test for drugs liable to be cholestatic using HepaRG hepatocytes

Christophe Chesné, Simone Khalil and Christiane Guguen-Guillouzo

Biopredic International, Saint Gregoire, France

customersinquiries@biopredic.com

Intrahepatic cholestasis is a chronic disease resulting from an impairment of the bile flow. This can lead to cirrhosis or severe hepatic insufficiency. Actually, a wide variety of commonly used drugs can induce cholestatic liver injuries. Unfortunately, the prediction of drugs liable to cholestatic is still poor. In this study, we develop a predictive screening test for drugs liable to cholestatic with HepaRG™ model using 2 complementary parameters, morphological alteration of bile canaliculi and impairment of bile flow. An assay to mimic cholestatic events was set up by treatment of HepaRG™ hepatocytes with several molecules known to be cholestatic including Chlorpromazine (CPZ), Fasudil (FSD) and Bosentan (BSN) as reference. The bile canaliculi alterations and bile acid flow rate were evaluated by using newly synthesized fluorescent bile acid derivatives. Imaging quantification was performed with the Cellomics ArrayScan™ . We first evidenced the bile canaliculi alterations known to be strictly associated with cholestasis, either constriction with CPZ or dilatation with FSD and BSN, and we evaluated the mean area by ZO-1 localization at the canalicular membrane or by using dextran-blue fluoroprobe; a factor 2 to 5 of variation was found compared to untreated cells. Then, new probes using first and secondary bile acids were tested for their efficacy. The conjugated forms such as urso-desoxycholate probe were the most specific and sensitive tool for evaluating drug-induced bile flow failure and to detect possible contribution of BSEP transporter. Moreover, CDFDA trafficking was used for evaluating MRP2 activity. The delayed trafficking in both CPZ-and BSN treated cells was associated with alteration of BSEP while the probe was abnormally accumulated in bile canaliculi in FSD-treated cells. In conclusion, the combination of HepaRG™ cell line with new predictive biomarkers is powerful for screening drug-induced cholestasis. In addition, the novel fluoroprobes provide new insights to early discriminate drugs acting by transporters alteration or canalicular junction disorders. This new assay represents a promising large-scale screening test for prediction of drugs liable to cholestasis potential.

III-456

I-screen: *In vitro* platform to study human gut microbiota induced drug metabolism and molecular transformations

Evita van de Steeg[1], Pascalle van Loo[1], Irene Nooijen[1], Scott Obach[2], Steven Erpelinck[1], Simon Folkertsma[1], Frank Schuren[1] and Wouter Vaes[1]

[1]TNO, Zeist, The Netherlands; [2]Pfizer, Inc., Groton, CT, United States

evita.vandesteeg@tno.nl

Our gut microbiome plays a major role in the metabolism of xenobiotics and is a relatively under-explored, but essential, field of study in pharmacology and toxicology. Gut microbiota can directly metabolize drugs into active, inactive or toxic metabolites, thereby influencing pharmacokinetics, efficacy and toxicity profiles of prescribed drugs. To study these processes *in vitro*, TNO has developed the i-screen platform, an intestinal screening multi-well system simulating the human colonic microbiota conditions. In the presented study, the metabolic capacity of human gut microbiota was investigated by incubating drugs potentially susceptible to microbial metabolism, under fully anaerobic conditions, in i-screen followed by LC-HRMS analysis of the samples. We identified different types of drug transformations that may occur in the human gut and are all known to occur *in vivo*. In conclusion, i-screen is a tool for *in vitro* evaluation of microbiota driven metabolism of drugs.

Innovative Models for Safety and Efficacy – Predictive Models

III-266

Organotypic culture models for predictive toxicology

Dusty Miller and Dave Cliffel

Chemistry, Vanderbilt, Nashville, TN, United States
dusty.r.miller@vanderbilt.edu

Regulatory agencies face the daunting task of determining whether any of the tens of thousands of commonly used chemicals pose a risk to human health and the environment. The prevailing environmental toxicology paradigm has relied on guideline animal studies that are expensive, pose troubling ethical questions, and are difficult to relate directly to human health hazards due to inter-species discordance.

To circumvent these problems, we use organotypic culture models (OCMs) as experimentally accessible systems of intermediate complexity to advance predictive toxicology. Integrating these OCMs with microphysiometry methods such as electrochemical detection of metabolites creates an amenable and affordable medium- to high-throughput toxicity screening model.

Using multianalyte microphysiometry, we can determine both the acute and chronic responses of multiple cell lines to exposures to drugs and other consumer-based chemicals. Our approach has the advantage of providing quantitative information regarding a variety of cellular activities, and hence can provide a new comprehensive approach to toxicological profiles.

III-276

Analysis of ToxCast data for food-relevant compounds by comparison with *in vivo* data using the RISK21 approach

Alexandra Turley[1,2,3], Kristin Isaacs[4], Barbara Wetmore[4], Agnes Karmaus[5], Michelle Embry[6] and Mansi Krishan[1]

[1]ILSI-North America, Washington, DC, United States; [2]Pharmacology and Toxicology, Michigan State University, East Lansing, MI, United States; [3]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI, United States; [4]U.S. Environmental Protection Agency, Research Triangle Park, NC, United States; [5]Integrated Laboratory Systems, Inc., Research Triangle Park, NC, United States; [6]ILSI-HESI, Washington, DC, United States
aturley@ilsi.org

The ToxCast program has generated a wealth of *in vitro* high throughput screening data, and best approaches for the interpretation and use of these data remain undetermined. We present case studies comparing the ToxCast and *in vivo* toxicity data for two food contact substances, sodium pyrithione and dibutyltin dichloride, using the RISK21 approach. Available exposure data, toxicity data, and model predictions were analyzed. *In-vitro* to *in-vivo* extrapolation (IVIVE) was performed to determine oral equivalent doses (OEDs) from ToxCast bioactivity. For sodium pyrithione, calculated OEDs corresponded to doses that demonstrated toxicity in animal studies. For dibutyltin dichloride, calculated OEDs were below the doses that demonstrated toxicity in animals, but this was confounded by the conservative estimates used in the IVIVE calculations. These studies highlight the potential of the ToxCast approach while also indicating areas where additional data or predictive tools are needed.

III-407

Predicting ToxCast and Tox21 bioactivity using toxprint chemotypes

Jeremy Fitzpatrick and Grace Patlewicz

National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park (RTP), NC, United States
fitzpatrick.jeremy@epa.gov

The EPA ToxCast™ and Tox21 programs have generated bioactivity data for nearly 9076 chemicals across ~1192 assay endpoints; however, for over 70% of the chemical-assay endpoint pairs there is no data. To help fill the gaps, we constructed random forest models for each assay endpoint, using chemotypes which are fragment based descriptors. For each model, the assay endpoint data was split into a training set containing 80% of the active chemicals and an equal number of in actives, with the remainder used as the test set. Many assay endpoints still lacked enough data to build effective models. The 277 models with at least 200 compounds in their training sets were effective at predicting bioactivity, with 250 of 277 generating predictions with greater than 60% balanced accuracy. Our models predict ToxCast™ and Tox21 bioactivity values in the absence of experimental data and may be useful for building predictive toxicity models.

This abstract may not reflect U.S. EPA views or policy.

III-449

Development of an acute oral toxicity dataset to facilitate assessment of existing QSARs and development of new models

Jeremy Fitzpatrick and Grace Patlewicz

National Center for Computational Toxicology (NCCT), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park (RTP), NC, United States
fitzpatrick.jeremy@epa.gov

Acute oral toxicity data are used to meet both regulatory and non-regulatory needs. Recently, there have been efforts to explore alternative approaches for predicting acute oral toxicity such as QSARs. Evaluating the performance and scope of existing models and investigating the feasibility of developing new models relies on a large set of curated acute toxicity data. We created a data set of rat oral LD_{50} values for16439 chemicals from a variety of sources. We used a subset of this dataset to: 1) evaluate LD_{50} predictions of two models TIMES and TEST, and 2) investigate the feasibility of developing new models using bioactivity data from Toxcast™ and Tox21. TIMES and TEST models had limited coverage. TIMES was able to make predictions for only 7% of the compounds tested, while TEST was able to make predictions for 23%. Our own ongoing modeling efforts have demonstrated a relationship between Toxcast™ assay results and acute oral toxicity.

This abstract may not reflect U.S. EPA policy.

III-474

Microphysiological system models and the Lautenberg Chemical Safety for the 21st Century Act

Barbara Klieforth[1], Rachel M. Shaffer[2], Marissa Smith[2] and Elaine Faustman[2]

[1]US EPA, Washington, DC, United States; [2]Department of Environmental and Occupational Health Sciences, University of Washington School of Public Health, Seattle, WA, United States
klieforth.barbara@epa.gov

The Frank R. Lautenberg Chemical Safety for the 21st Century Act, (the 2016 reauthorization of the Toxic Substances Control Act), states that the EPA must minimize the use of vertebrate animals for the testing of chemicals. The EPA must develop a strategic plan to promote the development and implementation of alternative test methods and testing strategies to generate information under TSCA that can reduce, refine, or replace the use of vertebrate animals, and fund and carry-out research, development, performance assessment, and translational studies to accelerate the development of those alternative test methods and strategies. Microphysiological systems and organotypic models, such as those pursued by the US EPA STAR Centers for Organotypic Culture Models for Predictive Toxicology(OCM-PTs), will undoubtedly be one such toxicity testing alternative valuable to the implementation of the Lautenberg Chemical Safety for the 21st Century Act.

III-569

Integration of biological analogy in the read-across approach for repeat-dose systemic toxicity assessment

Anne Riu, Françoise Gautier, Medhi Ez-Zoubir, Floriane Beaumard, Reine Note, Jacques Clouzeau and Silvia Teissier

L'Oréal, R&I, Aulnay sous Bois, France
ariu@rd.loreal.com

Read-across is a regulatory accepted strategy as alternative approach to animal testing for prediction of systemic toxicity of new cosmetic ingredients from existing data on similar chemicals. As developing read-across, integration of criteria other than structure comparison appeared necessary to fully justify chemical similarity. The present strategy, based on a multidisciplinary team, aims at identifying possible analogs and to justify the adequacy of the selected analogs for RDT prediction. Such an approach relies on structural alerts, physicochemical properties and ADME parameters for the target compounds and its analogs. This read-across pipeline is being enriched with new *in vitro* methods developed such as mitochondrial alterations to address acute and chronic toxicity and shed light on the relevance of "biological analogy". Here, one case study will be presented in order to evaluate the applicability and the added-value of these new methods integrating the "biological analogy" in addition to the structural analogy into this read-across strategy.

Innovative Models for Safety and Efficacy – Chemical and Drug Testing

III-334

Studies on oils and lipidic extracts: Innovative *in vitro* methods for risk and biological effects assessment

Patrice Rat[1], Teddy Leguillier[1], Annabelle L'Hermitte[2], Anne Rossignol-Castera[2], Anaïs Wakx[1], Mélody Dutot[1] and Elodie Olivier[1]

[1]Toxicology Laboratory, Paris Descartes University, Paris, France; [2]Oléos-Hallstar, Montpellier, France

patrice.rat@parisdescartes.fr

Oils and lipidic extracts are more and more used in cosmetic formulations. However, due to their lipophilic nature, safety and biological effects assessment is very difficult and requires dilutions in organic solvents that can modify their intrinsic properties. We developed a method that consists in incubating living cells with neat oils or lipid extracts for a short time followed by analysis techniques of high sensitivity and high specificity such as fluorescence cytometry or FRET. We used this method to study a vegetable oil from *Calophyllum inophyllum* and a lipidic extract from cork oak and coconut palm (Diam Oleoactif®). We were able to generate cytotoxicity results and data on their biological effects regarding inflammation and wound healing. This method constitutes an essential innovative tool for risk assessment and biological effects studies of oils and lipidic extracts.

III-471

An *in vitro* strategy for informing relative potency of chemicals acting through a common mode of action – Pyrrolizidine alkaloids

Catherine Mahony, John Troutman, Karen Blackburn, Cathy Lester, Stefan Pfuhler, Amy Roe and Sharon Stuard

Procter & Gamble, Mason, OH, United States

mahony.c@pg.com

Pyrrolizidine Alkaloids (PA) are found in certain plant families. There are hundreds of PAs but it is 1,2-unsaturated PAs that are relevant for safety assessment, as they are responsible for genotoxicity/carcinogenicity and hepatotoxicity via metabolic activation. Applying a single limit for a cumulative toxicity assessment is a highly conservative approach but is also an over-estimation of the risk, because the relative potencies with respect to toxicity differ as a consequence of structure differences. To derive robust relative potency factors (RPF) that can be used for risk assessment, an *in vitro* testing strategy will be employed to leverage extensive knowledge of the Mode of Action of PA's. Specifically, we will integrate *in vitro* measures of the genotoxic potential and daily body burden of PA-derived DNA adducts formed in HepaRG cells and hepatocytes to further elucidate the role of hepatic metabolism kinetics in relative potency determinations of PA's.

III-570

EPA's extramural grants research activities in advancing the alternative tools development to animal testing to support chemical evaluation

Santhini Ramasamy, Megan Christian, Meta Bonner and Khin Oo

US EPA, Washington, DC, United States

ramasamy.santhini@epa.gov

The National Research Council 2007 report presents a vision of the toxicity testing for the 21st century which utilizes the advances in toxicogenomics, bioinformatics, systems biology, epigenetics and computational toxicology to transform the whole animal based testing commonly accepted in the regulatory applications. The NRC committee envisioned that the predictive pathway-based assays will serve as the central component of a broad toxicity-testing strategy for assessing the biologic activity of new and existing compounds for which no adequate toxicity information is available. In realizing this vision, EPA has supported research both internally and through extramural grants program. This work identifies the tools and data gathered through extramural research grants in computational toxicology, identifies research data gaps and considers policy implications.

Reference

NRC – National Research Council (2007). *Toxicity Testing in the 21st Century: A Vision and A Strategy*. Washington, DC: The National Academies Press.

III-664

Advancements in pesticide safety assessment – Generating more relevant data with fewer animals

Sean Gehen[1], Marco Corvaro[2], Sabitha Papineni[1], Raja Settivari[3], Jyotigna Mehta[2] and Reza Rasoulpour[1]

[1]Dow AgroSciences LLC, Indianapolis, IN, United States; [2]Dow AgroSciences LLC, Abingdon, United Kingdom; [3]The Dow Chemical Company, Midland, MI, United States

scgehen@dow.com

Global registration of crop protection active ingredients and end-use products is a complex and often animal-intensive endeavor. Innovative strategies are needed to reduce animal use while providing the data needed to assess human risk. At Dow AgroSciences, several 3Rs-focused approaches are in place. First, endpoints historically evaluated in stand-alone studies are integrated into standard 28- or 90-day rodent toxicity studies (e.g. neurotoxicity, immunotoxicity, genotoxicity). Generation of toxicokinetic data (without use of satellite animals) provides a foundation for setting kinetically-derived dose-levels that avoid testing at non-relevant extreme doses. Finally, *in vitro* methods have recently been implemented as potential replacements for acute toxicity endpoints. To maximize the potential impact there is a need to accelerate globally harmonized approaches towards adoption of alternative methods.

III-777

Computational and cheminformatics approaches towards properties assessment of nanomaterials

Bakhtiyor Rasulev

Department of Coatings and Polymeric Materials, North Dakota State University, Fargo, ND, United States

bakhtiyor.rasulev@ndsu.edu

For the last two decades, breakthrough research has been going on in all aspects of materials science, including nanotechnology at an accelerated pace. New materials of unprecedented functionality and performance are being developed and characterized.

Here we show the adaptation and application of various computational and cheminformatics methods in functional materials / nanomaterials properties prediction, including physico-chemical, toxicological and pharmacological. Since nanomaterials are complex entities from chemical point of view the study of nanomaterials requires an interdisciplinary approach, involving multiple aspects ranging from physics and chemistry to biology and medicine. In this report, on several examples that were recently published, we show the successful cases of physico-chemical, pharmacological and toxicological properties predictions for nanomaterials. The discussed nanomaterials include metal oxides, metals, functionalized fullerenes and carbon nanotubes.

Innovative Models for Safety and Efficacy

III-14

Linking LRI AMBIT chemoinformatic system with the IUCLID substance database to support read-across of substance endpoint data and category formation

Bruno Hubesch
CEFIC LRI, Brussels, Belgium
bhu@cefic.be

Read-across and category formation are indispensable techniques in safety assessments of chemicals. The read-across approach is used on average in 20% of the Endpoint Study Records, while (Q)SAR is used in less than 1% of the dossiers, according to European Chemical Agency reports. Although many tools are available, only a limited number is capable to provide easily accessible data on substance identity, composition together with chemical structures and high-quality endpoint data.

The AMBIT software, funded initially within the CEFIC LRI programme, provides a web service and user-friendly web interface to a chemical database, various chemical structure search facilities and toxicity prediction models. The AMBIT data model was further extended to support substances with complex compositions and substances experimental data which allows importing data from the International Uniform Chemical Information Database (IUCLID) as well as other sources. Currently AMBIT supports manual upload of i5z files exported from IUCLID or semi-automatic import via IUCLID Web services. The chemical structures already contained in AMBIT are automatically linked to constituents/impurities/additives of the imported substances. The flexible data storage and visualization allows for user friendly presentation of study data (physicochemical properties, environmental fate, ecotoxicological and toxicological information) and composition. Comprehensive assessment workflows are developed for read-across and category formation based on all the data available in AMBIT. The assessment workflow facilitates the search for target and source structures, generating data matrices, gap filling and generating assessment reports with predefined formats automatically. The enhanced AMBIT facilitates drafting and improves quality for read-across and category formation and will be a useful tool for experts responsible for substance assessments.

III-172

Predicting *in silico* the Direct-Peptide-Reactivity-Assay (DPRA) within the Allergic Contact Dermatitis framework

Ann Detroyer[1], J. Eilstein[1], C. Piroird[1], S. Imbert[1], H. Ivanova[2], I. Popova[2], C. Kuseva[2], Y. Karakolev[2], S. Dimitrov[2] and Ovanes Mekenyan[2]
[1]L'Oréal R&I, Aulnay sous Bois, France; [2]Laboratory of Mathematical Chemistry, University "Prof. As. Zlatarov", Bourgas, Bulgaria
adetroyer@rd.loreal.com

Allergic Contact Dermatitis (ACD) depends, amongst other parameters, on the ability of chemicals to covalently bind with skin proteins. Thus, *in chemico* methods like Direct-Peptide-Reactivity-Assay (DPRA) were developed as one of the alternatives to the animal ACD tests, i.e. Local-Lymph-Node-Assay (LLNA).

In this context, mechanistically based *in silico* DPRA models were build, to potentially be used for the screening/design of new chemicals and to help evaluate experimental DPRA results at the tests' limits (low solubility chemicals, …).

Today, based on our DPRA and LLNA data variability study (Dimitrov et al., 2016), our models contain mechanistically justified Cysteine and Lysine peptide alerts at the 13% and 42% reactivity level with a 95% confidence. They are applied on chemicals and their oxidative derivatives generated by abiotic activation transformations, predicting the worst-case scenario. These models present good predictive performance and high transparency (justifications, applicability domain).

Reference

Dimitrov, S., Detroyer, A., Piroird, C. et al. (2016). Accounting for data variability, a key factor in in vivo/in vitro relationships: Application to the skin sensitization potency (in vivo LLNA versus in vitro DPRA) example. *J Appl Toxicol 36*, 1568-1578. doi:10.1002/jat.3318

III-293

Investigation of cell death mechanisms unleashed by two new ruthenium complexes in breast cancer cells

Elisângela Lacerda[1], Vivianne Velozo-Sá[1], Aliny Lima[1], Wanessa Carvalho[1], Francyelli Mello[1], Luciano Pereira[2] and Alzir Batista[2]

[1]Federal University of Goiás, Goiás, Brazil; [2]Federal University of São Carlos, São Paulo, Brazil

silveiralacerda@gmail.com

The aim of the present study was to investigate the cell death mechanisms of breast cancer cell lines treated with two ruthenium complexes, LGMC-68 and LGMC-69, respectively. First, we used MTT test to determine the IC_{50} values of breast cancer cells MDA-MB-231, MCF-7 and Ehrlich treated with both complexes for 48 h. We selected MDA treated as described above for further tests, because of its selectivity index when treated with LGMC-68(8.0). According to morphological test to detect apoptosis/necrosis, these complexes caused mainly apoptosis (34.33% and 29%). Cell cycle results by flow cytometry demonstrated a G0/G1 phase arrest (66.11% and 65.61%). AnnexinV/PI assay showed that MDA treated with LGMC-68 presented significant amounts of late apoptotic cells (23.39%). JC-1 assay showed that the percentage of cells with depolarized mitochondria increased from 10.2%-control to 25.2% and 35.3%, respectively. Thus, these complexes have been shown to be promising for breast cancer treatment.

Reference

Lima, A. P. Pereira, F. C., Almeida, M. A. et al. (2014). Cytoxicity and apoptotic mechanism of amino acid/ruthenium(II) complexes against sarcoma-180 tumor cells. *PLoS One 9*, e105865.

III-301

Metabolism and toxicity on *Daphnia magna* exposed to acetaminophen

Ryeo-Ok Kim, Mina Cho, Seokjoo Yoon and Woo-Keun Kim

System Toxicology Research Center, Korea Institute of Toxicology, Daejeon, South Korea

ryeook.kim@kitox.re.kr

Particularly, overdosing or prolonged use of drug involves clinical toxicity which is accentuated by the production of pharmacologically active metabolites. Therefore, understanding for the relationship between drug metabolism and reactive, toxic metabolites is important to predict the liver toxicity. *Daphnia magna* has been extensively studied in the aquatic ecology and toxicology because of suitable characteristics as an experimental model for manipulated and maintained in the laboratory, with its small size, simple body structure, short generation time and genetic homozygosity. We used acetaminophen as a target drug. As a result, adverse physiological responses and molecular regulations including GSH depletion, generation of toxic metabolites and ROS, activation of CYP and DNA repair genes were induced in acetaminophen exposure. We demonstrated that drug-metabolized mechanism of *Daphnia magna* is highly conserved in human despite of absence of the liver.

References

Borgatta, M., Hernandez, C., Decosterd, L. A. and Waridel, P. (2015). Shotgun ecotoxicoproteomics of Daphnia pulex: Biochemical effects of the anticancer drug tamoxifen. *J Proteome Res 14*, 279-291.

Oliverira, L. L., Antunes, S. C., Goncalves, F. et al. (2015). Evaluation of ecotoxicological effects of drugs on Daphnia magna using different enzymatic biomarkers. *Ecotoxicol Environ Saf 119*, 121-131.

III-324

A kinomics approach to safety testing: Towards an animal-free safety test for whooping cough vaccines

Cyrille Krul[1], Marc Teunis[1], Renate Nawrocki[1], Rob Ruijtenbeek[2] and Saertje Verkoeijen[1]

[1]University of Applied Sciences Utrecht, Utrecht, The Netherlands; [2]Pamgene, Den Bosch, The Netherlands

cyrille.krul@hu.nl

Whooping cough vaccines are routinely tested for absence of potentially harmful active pertussis toxin (PTx) before their release on the market. This is done using the distressing and lethal mouse histamine sensitization test. The *in vitro* alternative CHO cell clustering assay is a highly sensitive but subjective and non-quantitative assay with an incompletely understood mechanism. As part of the VAC2VAC consistency approach, we have previously demonstrated that human A549 cells show a similar morphological response to CHO cells when exposed to PTx. Next, we aim to identify molecular markers in these cells that can be used to detect the presence of PTx in whooping cough vaccines and intermediate products. To this end we will use kinomics to simultaneously identify the PTx-induced activity of > 100 tyrosine and serine/threonine kinases in both cell types. This will ultimately lead to the development of a novel *in vitro* assay able to quantify the presence of PTx in a simple and robust way, either in the sensitive CHO and/or in the relevant human A549 cells, leading to a large reduction of animal use in vaccine safety testing.

III-402

Profiling of innate immune responses to determine vaccine quality as part of the consistency approach

Jennifer Veth and Jeffrey Bajramovic

Alternatives, Biomedical Primate Research Centre, Rijswijk, The Netherlands

veth@bprc.nl

The efficacy of vaccines depends on their capacity to induce protective immune responses, which is traditionally tested in animals. Animal tests are not only regulatory required during vaccine development, but also prior to release of new batches of marketed vaccines. The EU-funded VAC2VAC consortium brings together public and private partners and aims to replace these repetitive animal tests with animal-free methodology. Rather than treating every vaccine batch as a new entity, each new batch will be extensively characterized for physicochemical, immunochemical and functional properties and compared to a safe and efficacious batch: the consistency approach.

As the efficacy of any given vaccine is at least in part dependent on the strength and the type of innate immune response provoked by the adjuvant/antigen combination in that vaccine, functional characterization of these responses can form a useful part of the consistency approach. Our lab has developed animal-free models that allow for the qualitative and quantitative assessment of innate immune signaling cascades. These models include libraries of human cell lines transfected with so-called pathogen pattern recognition receptors (Toll-like and NOD-like receptors) and engineered to express luciferase in response to NF-kappaB and interferon-responsive element-mediated signal transduction. The combined use of these bioassays in a tiered testing strategy leads to rapid, robust and detailed characterization of whole vaccines or of vaccine components and can also be used to sensitively detect possible endotoxin contamination when recombinant antigens are used. We will present our methodology as well as preliminary results using antigens, adjuvant components and whole vaccines.

III-537

Development of an alternative zebrafish model for intestinal and respiratory toxicity

Jae-Hak Park

Seoul National University, Seoul, South Korea

pjhak@snu.ac.kr

An evaluation of intestinal and respiratory toxicity is important because the gastrointestinal tract and respiratory system is the first barrier for oral xenobiotics and volatile substances. Until now, a rodent model has been recommended as the standard toxicity model and cell line has been used as an alternative to this model, but there are limitations regarding cost effectiveness and the need for mimicry of the human system. Zebrafish has a high similarity with mammalian intestine and lung. Thus, in this study, we investigated whether zebrafish has potential for use as an alternative model of toxicity test. Our results suggested that indomethacin induced a more pronounced and sensitive transcript level changes in CYP450 3A65, inflammation, and intestinal function in the zebrafish model than in the rat and cells models. The zebrafish exposed to PHMG-P, showed increase in mRNA levels of inflammation and fibrosis factors was correlated with collagen deposition in gill. Taken together, our results demonstrated that zebrafish could sufficiently reflect the intestinal and respiratory toxicity and have the potential as an animal model to evaluate these toxicities.

This study was supported by the Research Institute for Veterinary Science, BK21 PLUS Program for Creative Veterinary Science Research and KRIBB Research Initiative Program.

III-540

Validation of zebrafish as an alternative intestinal toxicity model using indomethacin, diclofenac and methotexate

Bokyeong Ryu[1], C-Yoon Kim[2], Ukjin Kim[1], Jin Kim[1], Cho-Rok Jung[3], Hanseul Oh[1], Jimin Lee[1] and Jaehak Park[1]

[1]Seoul National University, Seoul, South Korea; [2]Konkuk University, Seoul, South Korea; [3]Korea Research Institute of Bioscience & Biotechnology, Daejeon, South Korea

hobitmilk@naver.com

Intestinal toxicity assessment of new materials is fundamental in a developing process of new drugs because the gastrointestinal tract plays an important role in drug absorption. Traditionally, rodent models and *in vitro* models have been used as toxicity models for intestines despite the limitations including economic and ethical issues. Zebrafish has been proven a similarity in the aspects of anatomy and physiology. Thus, we evaluated whether zebrafish can be an alternative model of the intestinal toxicity test using traditionally used enterotoxicants including indomethacin, diclofenac and methotrexate in this study. Our results suggested that enterotoxicants induced similar reaction of mRNA expression level changes in intestinal function related genes, apoptosis indicators, oxidative stress markers and inflammation indicators with the rodent model and the *in vitro* model. The trends of histopathological changes in zebrafish and rodents were also similar. Our results demonstrated that zebrafish could have potential for being used as an alternative model for intestinal toxicity.

Theme IV – Sustainability

Coordinators
Eric Hutchinson, John Hopkins University, Baltimore, MD, United States
Pamela J. Spencer, ANGUS Chemical Company, Buffalo Grove, IL, United States

Oral Presentations

Session IV-1: Predictive Safety Approaches: Role in the Design of Inherently Safer Chemicals

Co-Chairs
Pamela J. Spencer, ANGUS Chemical Company, Buffalo Grove, IL, United States
Paul Carmichael, Unilever, Sharnbrook, United Kingdom

IV-1-593

An ounce of prevention is worth a pound of cure: Design of lower toxicity products using non-animal alternatives

Pamela Spencer
Angus Chemical Company, Buffalo Grove, IL, United States
pjspencer@angus.com

Historically, early identification and characterization of adverse effects of industrial chemicals was difficult because conventional toxicological test methods did not meet R&D needs (e.g. methods that are rapid, relatively inexpensive and amenable to small amounts of test material). Consequently, undesirable toxicological effects were identified closer to commercialization when little options for design changes exist and after significant investment of time, resources and money. For example, a 2-generation reproduction study cost more than $500,000, uses more than 3000 rats and takes 15 months to complete. Further time, money and resources are consumed in efforts to "defend and save" products identified to have adverse effects. Today, rapidly evolving, next generation safety assessment methodologies have the potential to transform how companies develop and commercialize new products and chemicals. New 21st century tools now make it feasible to incorporate toxicological assessments as early as the ideology stage of product development and to build in rules and criteria to guide the design of high efficacy/low toxicity compounds with many fewer animal tests. This presentation will describe the evaluation of several software packages to determine their ability to accurately predict mutagenicity, skin sensitization and ready biodegradability when compared to experimental results for nitroparaffin (NP) and NP-derivatives molecules and how the results were used to develop an early-stage screening evaluation for comparison of new molecules.

IV-1-165

Automated read-across for REACH

Anne Bonhoff
Chemistry, UL Environment, Schwerzenbach, Switzerland
Anne.Bonhoff@ul.com

A new predictive toxicology tool, REACHAcross, a collaboration between UL and researchers from Johns Hopkins University, will contribute strongly to reduced animal use.

REACHAcross was initially created using the publically available toxicology data from nearly 10,000 chemicals registered under REACH and has since been expanded to include toxicology data from other public databases. This highly dense chemical map allows finding similar molecules for most structures. The biological data available in these datasets combined with *in vivo* endpoints from REACH represent an enormous modeling potential: both chemical and biological similarity can be used to interpolate properties of structures. This approach is based strictly on the local validity of similar chemicals but offers a coverage of large parts of the chemical universe similar to a QSAR. It expresses the certainty of a prediction based on the local data around the substance of interest.

IV-1-576

Predictive safety approaches following ten years of TT21C: From inspiration to application

Paul Carmichael

Unilever, Sharnbrook, United Kingdom
paul.carmichael@unilever.com

What is a Next Generation Risk Assessment (NGRA)? Ten years ago the National Research Council was asked by the U.S. Environmental Protection Agency to review the state of the science and create a far-reaching vision for the future of toxicity testing. Today, through the application of these novel approaches, a NGRA becomes an exposure-led risk assessment solution to biological pathway-indicated hazard concerns. Indeed, since the publication of the inspirational book "Toxicity Testing in the 21st Century (TT21C): a Vision and a Strategy" many scientists from various disciplines, have been working to realise the challenge of how new technological advances in molecular biology and computational modelling can be brought together to make safety decisions without the need for animal testing.

Through extensive collaboration and evaluation of case study chemicals, we have arrived at a tiered approach that can adapt and improve. *In silico*-first approaches with informatics data-mining are initially used to identify pathways of concern and to formulate hypotheses for testing. Next, lead molecular initiating events (MIEs) and on/off-target pathways are evaluated with respect to relevant exposures through *in vitro* experimental data generation (transcriptomics, screening panels, high content imaging) teamed with computational modelling. Potentially, a final tier employing organotypic human cell models or more complex toxicokinetic/toxicodynamic computational models may be utilised to provide additional confidence in predictive safety decision making.

IV-1-206

The practical application of *in vitro* pulmonary models to assess acute and chronic effects of toxic exposure

Rodger Curren, Hans Raabe, Erin Hill, Mario Aragon and Holger Behrsing

Institute for In Vitro Sciences, Inc., Gaithersburg, MD, United States
rcurren@iivs.org

There is an increasing interest and directive for researchers to understand the dynamic aspects of pulmonary exposures to environmental chemicals, household products, and other inhaled materials. Accurate and dependable methods are needed to assess exposures that may lead to severe events such as pulmonary fibrosis and COPD. Important precursor events, e.g. goblet cell hyperplasia, collagen deposition, and impaired ciliary function can be detected *in vitro*. Tissue models which can inform on such changes include 3-dimensional (3D) reconstructed airways, spheroids of lung cell-origin, and *ex vivo* precision-cut slices. However, the complement of human-airway-specific cells and tissue architecture differs between models and this impacts how the biological responses must be interpreted. This presentation will review the benefits and uses of *in vitro* models, address areas for model improvement, and discuss how inhaled materials can be evaluated *in vitro* for their impact on human health.

IV-1-662

Evaluation of tiered testing strategies for assessing acute toxicity of multi-component mixtures

Raja Settivari[1], Sean Gehen[2], Marco Corvaro[3] and Sue Marty[1]

[1]Toxicology & Environmental Research & Consulting, Dow Chemical Company, Midland, MI, United States; [2]Predictive Safety Center, Dow AgroSciences LLC, Indianapolis, IN, United States; [3]Dow Chemical Services UK Ltd, Abingdon Oxon, United Kingdom
rssettivari@dow.com

The emerging alternative methods have demonstrated good promise to reduce and replace *in vivo* acute toxicity testing for single compounds. Effective implementation of the alternative methods to assess multi-component mixtures may have a substantial impact on reducing animal use and development of sustainable products, through their implementation in early product development stages. Herein, we present tiered testing strategies for assessing dermal and ocular irritation, a weight-of-evidence approach for skin sensitization and the Globally Harmonized System Acute Toxicity Estimate method for predicting systemic acute oral, dermal and inhalation toxicity of multi-component mixtures. The overall concordance of these testing methods compared to existing *in vivo* data ranged from 75-95% across endpoints. Taken together, selected alternative methods demonstrated promising predictions for evaluating acute toxicity of multi-component mixtures.

IV-1-501

An animal free roadmap for agrochemical formulations safety assessment – Part 2: Non-testing and regulatory strategies

Marco Corvaro[1], Sean Gehen[2], Raja Settivari[3] and Jyotigna Mehta[1]

[1]Dow Chemical Services UK Ltd, Abingdon Oxon, United Kingdom; [2]Dow AgroSciences, Indianapolis, IN, United States; [3]Dow Chemical Company, Midland, MI, United States
mcorvaro@dow.com

Testing and non-testing evidence for agrochemical formulation was reviewed for regulatory applicability within the context of OECD Integrated Approach on Testing and Assessment (IATA). Analysis of the prevalence of health effects in large scale databases is key in defining relevant testing strategies for regulatory use. A retrospective analysis of *in vivo* acute studies from > 220 agrochemical formulations allowed identification of endpoint of concern, interrelation between endpoints and testing prioritisation strategy. The reciprocal predictivity of acute systemic endpoints between acute oral and acute dermal/inhalation and irritation endpoints between skin *and* eye irritation combined with good predictivity of the GHS additivity calculation approach (> 85-90%) provided valuable evidence for non-testing strategies. Furthermore, this evidence consolidated with the available *in vitro* testing strategies (reviewed in poster Part 1 – Settivari) could be a basis for defining an animal-free regulatory testing strategy and still allow identifying further research needs for agrochemicals.

IV-1-354

Towards a Lifecycle based Chemical Alternatives Assessment (LCAA)

Olivier Jolliet[1], Peter Fantke[2] and Lei Huang[3]

[1]University of Michigan, School of Public Health, Environmental Health Sciences, Ann Arbor, MI, United States; [2]Technical University Denmark, Lyngby, Denmark; [3]Environmental Health Sciences, University of Michigan, School of Public Health, Ann Arbor, MI, United States
ojolliet@umich.edu

There is a need for an operational life-cycle based quantitative screening-level assessment able to serve both Life cycle Assessment (LCA) and chemical alternatives assessment (CAA). This presentation therefore develops a "Life Cycle Based Chemical Alternative Assessment (LCAA)" and illustrates it though a proof-of-concept case study of two alternative plasticizers in vinyl flooring (DEHP vs DIHP).

The proposed LCAA combines: a) a manufacturing chemical inventory – based on the environmental genome of industrial products, b) near-field exposure to consumer products during use – based on first-order inter-compartmental transfer fractions to determine Product Intake Fractions, and c) toxicity-related outcomes – compared with other life cycle impacts.

First order releases of DEHP and DIHP are restricted, less than 2% over 3 years. For climate change, there is little difference between the two plasticizers, whereas compared to DEHP, DIHP impacts are reduced by a factor 10 for human health and a factor 3 for ecotoxicity. This study demonstrates the feasibility of the LCAA approach and the importance of considering consumer exposure during product use.

IV-1-824

A tiered approach to *in vitro*-based safety assessments: A case study on using computational modeling and fit-for-purpose *in vitro* assays to evaluate zones of safe exposure with xenoestrogens

Rebecca Clewell, Daniel Doheny, Kamel Mansouri and Patrick McMullen

ScitoVation, Durham, NC, United States
rclewell@scitovation.com

Catalyzed by the NAS reports Toxicity Testing in the 21st Century (2007) and Exposure Science in the 21st Century (2011), the regulatory and scientific communities are developing safety assessment strategies that employ computational models and *in vitro* human cell based assays. We are developing a tiered testing strategy that fundamentally incorporates *in silico* and *in vitro* technologies into the risk assessment paradigm. This tiered approach moves from lower tiers focused on rapid decision making and prioritization to higher tiers with increased biological complexity focused on improving accuracy and providing the necessary dose-response information for making chemical safety decisions. Tier 0 uses high-throughput computational models to predict exposure and chemical bioactivity for rapid chemical triage and prioritization for testing. Tier 1 uses rapid screening of compound bioactivity (e.g., ToxCast), together with high-throughput metabolism, HT-IVIVE and estimates of exposure, to develop data-driven margins of exposure (MoEs). Tier 2 uses fit-for-purpose *in vitro* assays designed to recapitulate *in vivo* human response to conduct in-depth dose-response evaluation of key toxicity pathways. Coupled with human relevant metabolism and quantitative IVIVE (Q-IVIVE), which accounts for metabolite generation and bioactivation, these Tier 2 dose-response studies are expected to support prediction of regions of safety – or exposure concentrations at which no increased risk is expected in humans. To support such decisions, Tier 2 assays should recapitulate not only a particular *in vivo* phenotype, but also the human relevant concentration-response for chemical effects. This presentation describes the process of developing and validating such fit-for-purpose *in vitro* assays. Finally, we demonstrate application of the tiered approach using a case study with estrogenic compounds.

Session IV-2: Approaches and Frameworks to Advance Alternative Analysis

Co-Chairs
Lauren Heine, Northwest Green Chemistry, Spokane, WA, United States
Margaret Whittaker, ToxServices LLC, Washington, DC, United States

IV-2-748

Alternatives assessment as an instrument for chemicals policy and product development

Lauren Heine[1] and Margaret Whittaker[2]
[1]Northwest Green Chemistry, Spokane, WA, United States; [2]ToxServices LLC, Washington, DC, United States
lheine@northwestgreenchemistry.org

Alternatives Assessment (AA) is increasingly used as a policy instrument and science-based process for identifying, comparing, and selecting, or initiating new development of safer alternatives to chemicals of concern (including those in materials, processes, or technologies) on the basis of their hazards, exposure potential, performance, and economic viability. Guiding frameworks for AA have been developed by the National Research Council and the Interstate Chemicals Clearinghouse. Using these frameworks, Northwest Green Chemistry is currently evaluating alternatives to copper-based antifouling paints in support of legislation by WA State to ban copper in recreational boat paints. This session will describe how alternative technologies are scoped, assessed and compared for disparate technologies ranging from those based on alternative biocides in paints, to hard barrier coatings to ultrasound and low frequency vibration. Challenges and lessons learned from this case study will be shared as well as decision frameworks.

IV-2-749

Toxicology in alternatives assessment: Evaluating individual chemicals, mixtures, and addressing data gaps

Margaret Whittaker[1] and Lauren Heine[2]
[1]ToxServices LLC, Washington, DC, United States; [2]Northwest Green Chemistry, Spokane, WA, United States
Mwhittaker@toxservices.com

Chemical hazard assessment (CHA) is a critical element of alternatives assessment. Decisions about inherently safer alternatives are made within the context of how chemicals are used, potential for exposure to humans and the environment, cost and availability, and life cycle impacts. The goal is to move away from chemicals of concern in products to options that do not lead to regrettable substitutions. Assessors are faced with challenges starting with obtaining full ingredient disclosure from suppliers in order to assess individual chemicals in products. In lieu of full disclosure, whole product testing strategies may be implemented, ideally, without resorting to animal testing. Even when ingredient disclosure is obtained, assessors may still be faced with a paucity of toxicity data. In order to fill data gaps to assess a product against multiple hazard endpoints, assessors must integrate many types of hazard data, using *in vitro*, *in silico*, and read-across methods and compare whole products and mixtures based on hazards of individual chemicals. Case studies applying such an integrated approach will be presented, and demonstrate that it is indeed possible to avoid regrettable substitution during the product design process.

IV-2-761

Framework for alternative analysis: Metabolism-induced toxicity assays on a 384-pillar plate

Moo-Yeal Lee and Rayton Gerald
Chemical & Biomedial Engineering, Cleveland State University, Cleveland, OH, United States
m.lee68@csuohio.edu

There is a critical need for incorporating physiological levels of chemical metabolism into high-throughput screening to lower therapeutic drug candidate attrition and to exclude potential environmental toxicants from the public domain. As a participant in EPA's "The Transform Tox Testing Challenge: Innovating for Metabolism", we developed a 384-pillar plate, complementary to conventional 384-well plates, for high-throughput, high-content, metabolism-induced toxicity assays. As a proof of concept, HEK293 cells in alginate-Matrigel were printed on the 384-pillar plate for miniaturized 3D cell culture, which were exposed to model compounds and drug metabolizing enzymes (DMEs) in a 384-well plate. The toxicity of the compounds and their metabolites generated *in situ* by the DMEs were successfully assessed by staining HEK293 cells with calcein AM and CellTiter-Glo® luminescent cell viability kit and determining augmented toxicity and detoxification of the compounds on specific DMEs. In summary, the 384-pillar plate generated metabolism-induced toxicity data necessary to establish *in vitro*/*in vivo* correlations with high predictability.

IV-2-743

Utilisation and comparison of *in vitro* skin models for the evaluation of dermal drug metabolism

Rachel Upcott-Gill

Drug Metabolism, Cyprotex, Kalamazoo, MI, United States
R.UpcottGill@cyprotex.com

Evaluation of the potential metabolism of dermally applied pharmaceuticals in the skin is especially important for drugs and chemicals designed for topical application because of the risk that protein reactive metabolites formed in the skin may contribute to, or cause, skin sensitisation. In addition, evaluating skin metabolism can give insight into the potential impact on skin absorption of test compounds. Metabolism in the skin may alter the physicochemical properties of a pharmaceutical, increasing its absorbance through the skin and may impact on the systemic availability of both parent drug and metabolite.

Whilst various reconstructed or full thickness 3D models are morphologically similar to native human skin and offer comparable functionality, the use of more simple *in vitro* models such as human skin S9 or the immortalised human keratinocyte cell line, HaCaT, could present a viable, low-cost alternative that could provide relevant data comparable with more complex approaches.

This presentation provides an overview as to why skin metabolism is of importance and also focuses on our experience in characterising *in vitro* metabolism in a range of skin models. We have focused on evaluating whether *in vitro* systems for skin metabolism can provide an assessment of bioactivation potential or metabolic liability. We have also evaluated the differences between hepatic and cutaneous metabolism. The initial aim was to characterise metabolic capability using archetypal enzyme-specific substrates for Phase I and Phase II metabolism. In addition, we have evaluated a range of compounds known to cause skin sensitisation after bioactivation. The *in vitro* models we have evaluated are human skin S9 fractions, which contains human cytosolic and microsomal enzymes, human liver S9, and the immortalised human keratinocyte cell line HaCaT. We also evaluated the use of high content screening (HCS) approaches to identify evidence of bioactivation in HaCaT cells by investigating glutathione conjugation in HaCaT cells.

The metabolic competency of the skin differs vastly from the hepatic systems. Overall HaCaT cells show a higher metabolic capacity than skin S9 derived from epidermis and dermis. However, some specific enzymes such as aldehyde oxidase were found to have higher activity in skin S9. Flavin-containing monooxygenase (FMO) and esterases were detected in all three *in vitro* systems evaluated. Higher formation of the N-acetylated metabolite of 4-aminobenzoic acid (PABA), was observed in HaCaT cells, and the glucuronidation of multiple substrates was also observed in HaCaT. Metabolism of testosterone was also evaluated in these systems as a CYP3A probe substrate. Despite the absence of evidence for oxidation of this substrate in HaCaT cells, specific reduction of testosterone to form dihydrotestosterone was observed indicating 5α-reductase activity in this cell type. This suggests a potential steroid metabolic pathway in this skin model.

Overall, we demonstrate differential metabolism between the different simple *in vitro* systems and discuss the potential suitability of such models in evaluating skin metabolism. However, there are clear limitations with respect to overall enzyme levels in these *in vitro* systems. 3D alternatives have been developed for safety assessment purposes, and the potential advantages of utilising 3D models for assessing skin metabolism will also be discussed. This approach for evaluating and understanding the potential for skin metabolism is also relevant to the Cosmetic Europe Skin Tolerance Task Force testing strategy for skin sensitisation potency prediction, and should help in introducing bioavailability and metabolism into an integrated approach for skin safety assessment.

IV-2-831

The intersection between LCA and Green Chemistry in California's Safer Consumer Products regulations

Meredith Williams, Lynn Nakayama-Wong, Jim Polisini, Vivek Mathrani and Jeff Wong

Department of Toxic Substances Control, Sacramento, CA, United States
Meredith.Williams@dtsc.ca.gov

California's Safer Consumer Products (SCP) regulations require manufacturers to pursue safer alternatives for products determined to have the potential for harm and listed as Priority Products. Priority Products are product-chemical combinations where there is a potential for exposure to one or more hazardous chemicals in the product. The narrative standard product prioritization and the objective to identify "safer" alternatives creates a decision-making framework distinct from traditional risk assessment. Implications of these differences will be discussed.

In the SCP Alternatives Analysis framework, alternatives are evaluated using elements of hazard assessment, exposure analysis, and LCA. Many tools exist to perform an Alternatives Analysis as documented in the California Alternatives Analysis Guide. Full integration of these approaches remains challenging. Chemicals developed using Green Chemistry principles are likely to compare favorably in chemical hazard assessment and LCA. This presentation will explore links between Green Chemistry and the Alternatives Analysis framework and identify opportunities to embed Green Chemistry in Alternatives Analysis.

Session IV-3: Educating and Training Scientists to be Future Leaders in Sustainability

Co-Chairs
Amy Cannon, Beyond Benign, Wilmington, MA, United States
Saskia van Bergen, Washington State Department of Ecology, Olympia, WA, United States

IV-3-786

Toxicology for chemists: Connecting toxicology and chemistry topics for the design of chemical products that have reduced human and environmental impacts

Amy Cannon
Beyond Benign, Wilmington, MA, United States
Amy_Cannon@beyondbenign.org

There is a movement towards teaching toxicology concepts within chemistry courses and programs to better prepare students with the skills necessary to design chemical products and processes with reduced environmental and health hazards. Chemistry programs today are currently void of any training or course work on toxicology and related subjects. There is growing demand for teaching toxicology and related subjects to chemistry students, with the goal of providing students with the skills to design chemical products and processes that have reduced hazards. Beyond Benign, a non-profit organization dedicated to green chemistry education, facilitates a working group comprised of chemistry faculty and professional toxicologists that is working towards developing resources and curriculum to enable faculty to engage with their students on toxicology topics. This presentation will discuss the ongoing work within the toxicology working group and provide examples of courses and programs where concepts are being integrated in to chemistry programs. With a greater understanding of toxicological principles, and related subjects, the next generation of molecular designers can be poised with the skills to design chemical products that are safer and more sustainable.

IV-3-764

A recipe for prevention: Embedding environmental health in healthcare

Patrice Sutton, Robert Gould, Marya Zlatnick, Naomi Stotland, Julie Lindow, Hannah Archibald, Nikita Sinha, Jessica Kim, Annemarie Charlesworth and Tracey Woodruff
University of California San Francisco, San Francisco, CA, United States
Patrice.Sutton@ucsf.edu

To integrate environmental health science into health professional learning we undertook a collaborative project to embed environmental health within medical professional curriculum and other learning opportunities.

Results: We developed and implemented an elective and a syllabus for the *Reproductive Environmental Health* module for the second-year medical students; determined best opportunities to embed environmental health throughout 2nd year core curriculum module; participated in the UC-wide *Carbon Neutrality Initiative*, which brought together UC professors to identify ways to incorporate climate change in UCSF's curriculum; disseminated CME opportunities; published in a leading medical text book and articles in medical professional journals; and initiated organizing a nascent group of academics across the U.S. interested in working together on this issue.

Conclusion: These successes can serve as models for healthcare institutions across the country.

Reference

http://prhe.ucsf.edu/sites/prhe.ucsf.edu/files/December-16.pdf

IV-3-768

Educational tools for designing safer chemicals and predicting toxicity

Terrance Kavanagh[1], N. J. Simcox[1], G. A. Lasker[1], K. E. Mellor[2], M. L. Mullins[3], S. Nesmith[3], E. P. Gallagher[1], M. Mills[1], D. Botta[1], S. C. Schmuck[1], P. Coish[2], B. W. Brooks[3], J. Corrales[3], A. Voutchkova-Kostal[4], J. Kostal[2], L. Kristofoco[3], G Saari[3], W. B. Steele[3], L. Q. Shen[2], F. Melnikov[2] and J. Zimmerman[2]

[1]University of Washington, Seattle, WA, United States; [2]Yale University, New Haven, CT, United States; [3]Baylor University, Waco, TX, United States; [4]George Washington University, Washington, DC, United States
tjkav@uw.edu

There are over 100,000 registered chemicals in commerce in the US with over 700 new chemicals introduced every year. Many of these chemicals lack toxicity data that is expensive and time-consuming to collect. To address these issues, the Molecular Design Research Network (MoDRN) is developing tools for chemical assessment and design based in computational chemistry and predictive toxicology and reduce, and eventually replace, animal testing with *in vitro* and *in silico* tools. MoDRN is a collaboration between chemists, toxicologists, computational toxicologists, and biologists from four different universities. A significant part of this collaboration is to educate the next generation scientists and practitioners in the nexus of chemistry and toxicology and familiarize audiences with the concept of designing safer chemicals. This session will describe the principles of chemical design, introduce design tools, and highlight the education and outreach materials developed by MoDRN.

IV-3-770

What role can state agencies play in mainstreaming Green Chemistry education efforts?

Saskia van Bergen

Hazard Waste and Toxics Reduction, Washington State Department of Ecology, Olympia, WA, United States
Saskia.vanBergen@ecy.wa.gov

Teaching students to become leaders in sustainability requires the right tools, training, and the ability to engage students.

It seems unlikely that a regulatory state agency would play a role in developing future leaders in sustainability. However, our agency's diverse work creates a broad range of partnerships than enable us to collaborate with many different groups, leverage efforts, and promote tools, curriculum, and trainings. Learn how the Department of Ecology is developing tools and helping educators and companies incorporate green chemistry and safer alternatives.

Session IV-4: The 3Rs and One Health: From Environment to Farm to Medicine

Co-Chairs
Alan Goldberg, Johns Hopkins University, Baltimore, MD, United States
Meghan Davis, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

IV-4-803

Canary with a fever: Natural animal models for zoonotic infectious disease

Peter Rabinowitz
Environmental and Occupational Health Sciences, University of Washington Center for One Health Research, Seattle, WA, United States
peterr7@uw.edu

More than two thirds of emerging infectious diseases are zoonotic (transmitted between animals and humans) in origin. The majority of recent emerging zoonoses have wildlife reservoirs. (Palmer et al., 2017) Despite their growing importance, scientific understanding of many zoonotic diseases lags behind that of many other diseases, as does development of effective diagnostics, therapeutics, and vaccines. Traditional experimental animal models have inherent limitations in researching zoonotic diseases due to the diversity of types of pathogens and the fact that many zoonotic pathogens are host specific (Yugo et al., 2014), and often are found primarily in wildlife. The sheer diversity of zoonotic pathogens (viral, bacterial, parasitic, fungal) argues for greater use of natural animal models. Variations in resistance and susceptibility to a specific pathogen within and between species can be explored through whole genome sequencing and other molecular approaches to identify targets for novel therapeutics and vaccines. Since zoonotic diseases threaten both human and animal populations, such discoveries hold potential for improving the health of both humans and non-human animals. Examples of this concept will be presented.

References

Palmer, M., Cox, R. J., Waters, R. W. et al. (2017). Using white-tailed deer (odocoileus virginianus) in infectious disease research. *Am Assoc Lab Anim Sci*, Epub ahead of print.

Yugo, D. M., Cossaboom, C. M. and Meng, X. J. (2014). Naturally occurring animal models of human hepatitis E virus infection. *ILAR J 55*, 187-199. doi:10.1093/ilar/ilu007

IV-4-669

The consequences of industrial farm animal welfare on human health

Alan Goldberg
Department of Environmental Health & Engineering, Johns Hopkins University, Baltimore, MD, United States
agoldbe1@jhu.edu

This paper will explore the relationships between farm animal welfare, industrial farm animal production, and human health. The data suggest that when animal welfare of land-based farm animals is compromised through inhumane intensification, there are resulting significant negative human health consequences due to environmental degradation and the use of non-therapeutic levels of antibiotics for growth promotion/disease prevention. This paper accepts that even if animal based protein consumption is reduced, meat and fish will be part of the diet of the future. Industrial production, modified from the current inhumane intensified systems, will still be required to feed the world in 2050 and beyond. This paper identifies the concept of sustainable intensification and suggests that if farm animal welfare is improved, many of the human health consequences of intensified industrial production can be eliminated or reduced.

IV-4-752

Occurrence of *Staphylococcus aureus* from swine and swine workplace environments on industrial and antibiotic-free hog operations in North Carolina, USA: A One Health pilot study

Meghan Davis[1], *Nora Pisanic*[1], *Sarah Rhodes*[2], *Alexis Brown*[1], *Ana Rule*[1] *and Christopher Heaney*[1]

[1]Department of Environmental Health & Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States; [2]Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, United States
mdavis65@jhu.edu

Industrial swine production has been linked to risk of antibiotic-resistant strains of *Staphylococcus aureus* in hogs and workers. We evaluated 1 operation that raised swine in confinement with antibiotics (industrial hog operation: IHO) versus 3 on pasture without antibiotics (antibiotic-free hog operation: AFHO). Hog, ambient air, and worker surrogate personal air samples were analyzed for *S. aureus*; isolates were tested for antimicrobial resistance, absence of scn (livestock marker), and spa type. *S. aureus* was recovered from 17/20 (85%) hogs, 8/14 (67%) ambient air and both (100%) personal air samples at the IHO; no *S. aureus* isolates were recovered from 30 swine, 19 ambient and 6 personal air samples at the 3 AFHOs. All *S. aureus* recovered from IHO hogs, ambient air, and personal air were scn negative and spa type t337; 62/63 (98%) were multidrug resistant. Larger studies are needed to relate operation practices, pathogen exposures, and animal or human health outcomes.

IV-4-758

Gene editing livestock: Part of the solution or part of the problem?

Adam Shriver

Centre for Applied Ethics, University of British Columbia, Vancouver, BC, United States
adam.shriver@ubc.ca

Current intensive agriculture practices raise ethical concerns related to human health, the environment, and animal welfare, all against a backdrop of a growing global population. Some have suggested that new gene editing technologies associated with CRISPR/Cas9 could help address many of these concerns. However, the initial reaction of the public to such proposals is often decidedly negative. In this presentation, I will review the various ways gene editing could potentially be used to address problems with intensive agriculture, and will examine the new ethical concerns raised by the genetic modification of animals.

IV-4-823

Beyond the mouse trap: A look at canine atopic dermatitis as a naturally occurring model for human disease

Jennifer Gardner

Dermatology, University of Washington, Seattle, WA, United States
jen1110@uw.edu

Transgenic mouse models have been utilized in experimental settings to understand correlates of human disease. Though they can provide a basic understanding of the pathophysiology of a disordered state, this does not always translate from "bench to bedside" seamlessly. For one thing, mice do not naturally suffer from many of the same diseases as humans, so they are artificially engineered to express the phenotype that resembles a human disease. Additionally, lab mice do not share the same environment as humans, so the environmental stresses that contribute to disease states in humans are not taken into account in the mouse model paradigm.

An alternative, and potentially more enlightening, approach comprises looking to the animal world at spontaneously occurring disorders that are homologous to those seen in humans. Companion animals, or pets, often share the same environment as their humans. Thus, looking to the veterinary world to understand the pathophysiology and treatment experience of such disorders can be applicable to human medicine. One such naturally occurring disease is that of atopic dermatitis in canines (cAD) and humans (hAD). We will discuss some of the shared features of cAD and hAD with regard to clinical presentation, skin barrier function, immunology and the cutaneous microbiome. This type of comparative work opens up possibilities for research using a canine model of AD to gain insight into new approaches to treating human skin conditions based on the experience of veterinary dermatology. Additionally, a collaborative research approach looking at spontaneously occurring animal models of human disease in companion animals has great potential in advancing therapies to improve the health of both species.

References

Bradley, C. W., Morris, D. O., Rankin, S. C. et al. (2016). Longitudinal evaluation of the skin microbiome and association with microenvironment and treatment in canine atopic dermatitis. *J Infect Dis 136*, 1182-1190.

Kong, H. H., Oh, J., Deming, C. et al. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res 22*, 850-859.

Marsella, R., Sousa, C. A., Gonzales, A. J. and Fadok, V. A. (2012). Current understanding of the pathophysiologic mechanisms of canine atopic dermatitis. *JAVMA 241*, 194-207.

Marsella, R. (2013). Fixing the skin barrier: Past, present and future – Man and dog compared. *Vet Dermatol 24*, 73-76.

Santoro, D., Marsella, R., Pucheu-Haston C. M. et al. (2015). Pathogenesis of canine atopic dermatitis: Skin barrier and host-micro-organism interaction. *Vet Dermatol 26*, 84-94.

Santoro, D. and Rodrigues Hoffmann, A. (2016). Canine and human atopic dermatitis: Two faces of the same host-microbiobe interaction. *J Infect Dis 136*, 1087-1089.

Saridomichelakis, M. N. and Olivry, T. (2016). An update on the treatment of canine atopic dermatitis. *Vet J 207*, 29-37.

Session IV: Sustainability

Poster Presentations

IV-119

Genetically modified animals killed in stock

Marjolein Schilders-van Boxel, Jan-Bas Prins, Herman Koëter, Henriëtte Bout, Coenraad Hendriksen, Wim de Leeuw, Reineke Hameleers, Pieter Roelfsema, Frauke Ohl †, Frank Dales, Leane van Weereld and Diane Kegler

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands

Marjolein.vanboxel@rvo.nl

Animals killed in stock does not only imply surplus animals from genetic modification projects: approximately 25% of the animals killed before the procedure are non-genetically modified animals. The substantial increase of animals killed in stock is primarily due to experiments with zebrafish and mice. The main reason for killing these animals is that they have an unsuitable genetic composition. Other reasons include age, weight, or incorrect gender for the research purposes.

The advisory report "Genetically modified animals killed in stock" was drawn up by the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) at the request of the Minister for Agriculture. The report focuses primarily on reducing the number of genetically modified (GM) animals that "died or killed in stock", especially fish and mice. However, the NCad also draws attention to other aspects that it considers relevant to the dynamics of the numbers and species of GM animals used. It also places the issue of the number of animals killed in stock in a broader perspective.

Recent years have seen the development of "genome editing", a new and innovative technology that can be used to create genetically modified animals. Genome editing is increasingly replacing traditional transgenic technology. This new technology is expected to make it possible to create a genetically altered animal that is tailor-made for a given experiment while using fewer animals in the process than has hitherto been the case. The advisory report builds on previous initiatives by the Ministry of Economic Affairs and was published in December 2015. This poster will give an overview of the advisory report by the NCad and the actions taken by the stakeholders ever since.

Reference

https://www.ncadierproevenbeleid.nl/documenten/rapport/2015/11/26/advise-stock-animals

IV-255

Characterization of an equine 3D *in vitro* fracture hematoma

Annemarie Lang[1,2,3], Janika Bartsch[1], Moritz Pfeiffenberger[1,3,4], Frank Buttgereit[1,3] and Timo Gaber[1,3]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Berlin-Brandenburg School of Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany; [3]German Rheumatism Research Centre, Berlin, Germany; [4]Dahlem Research School, Freie Universität Berlin, Berlin, Germany

annemarie.lang@charite.de

Fracture healing is a complex process, starting with an inflammatory phase driven by a hypoxic microenvironment. The initial phase or fracture hematoma (FH) has been proven to be crucial for an optimal healing success. Different animal models are used to clarify the underlying pathways. With respect to comparative medicine and the increasing expenses of the horse industry for repair and rehabilitation of fractures, we developed an *in vitro* equine FH model. Therefore, equine blood was mixed and clotted with equine mesenchymal stromal cells in a 96-well-plate to generate *in vitro* FHs. FHs are cultivated in 48-well-plates under normoxic and hypoxic conditions for 6 h, 12 h, and 48 h. Subsequently, the hematoma has been characterized by flow cytometry and gene expression. As a result, we observed great similarities between the equine FH and the human FH in terms of cell composition while the mRNA-expression indicate clear differences in the fracture healing process between horses and humans.

IV-316

Establishment of clival chordoma cell line MUG-CC1 and lymphoblastoid cells as a model for potential new treatment strategies

Beate Rinner, Verena Gellner, Peter Valentin Tomazic, Birgit Lohberger, Katharina Meditz, Ellen Heitzer, Michael Mokry, Andreas Leithner, Birgit Reininger-Gutmann and Bernadette Liegl-Atzwanger

Medical University of Graz, Graz, Austria

beate.rinner@medunigraz.at

Important tools for 3Rs are predictive, physiological relevant cell culture systems. Chordomas are rare malignant tumors that develop from embryonic remnants of the notochord and arise only in the midline from the clivus to the sacrum. Surgery followed by radiotherapy is the standard treatment. As chordomas are resistant to chemotherapy, further treatment options are urgently needed 1. MUG-CC1 is strongly positive for brachyury, cytokeratin, and S100. The cell line showed gains of the entire chromosomes 1, 7, 8, 12, 13, 16, 17, 18, and 20. During chordom cell line cultivation a spontaneous lymphoblastoid EBV-positive cell line grew out, we characterized the suspension cells in detail. The immortalized lymphoblastoid cell line MUG-CC1-LCL provides material for a variety of assays and will be used as a source of biomolecules like DNA, RNA, and proteins.

A new, well-characterized clival chordoma cell line, as well as a non-tumorigenic lymphoblastoid cell line should serve as an *in vitro* model for the development of potential new treatment strategies for patients suffering from this disease and to further understand the pathogenesis and tumor biology of chordomas.

Reference

Stacchiotti, S. and Sommer, J. (2015). Chordoma Global Consensus Group. Building a global consensus approach to chordoma: A position paper from the medical and patient community. *Lancet Oncol 16*, e71-83.

IV-320

MUG-Mel2, a novel highly pigmented and well characterized NRAS mutated human melanoma cell line

Beate Rinner[1], Greta Gandolfi[2], Katharina Meditz[1], Marie-Therese Frisch[1], Karin Wagner[1], Alessia Ciarrocchi[2], Federica Torricelli[2], Raili Koivuniemi[3], Johanna Niklander[3], Bernadette Liegl-Atzwanger[1], Birgit Lohberger[1], Nassim Ghaffari-Tabrizi-Wizsy[1], Ellen Heitzer[1], Dagmar Zweytick[4], Birgit Reininger-Gutmann[1] and Iris Zalaudek[1]

[1]Medical University of Graz, Graz, Austria; [2]Laboratory of Translational Research, Arcispedale S. Maria Nuova-IRCCS, Reggio Emilia, Italy; [3]University of Helsinki, Helsinki, Finland; [4]Institute of Molecular Biosciences, Biophysics Division, NAWI Graz, University of Graz, Graz, Austria

beate.rinner@medunigraz.at

Important tools for 3Rs are predictive, physological relevant cell culture systems. NRAS mutation in melanoma has been associated with aggressive tumor biology and poor prognosis. Although targeted therapy has been tested for NRAS mutated melanoma, response rates still appear much weaker, than in BRAF mutated melanoma. While plenty of cell lines exist, only few melanogenic cell lines retain their *in vivo* characteristics. We present an intensively pigmented and well-characterized cell line derived from a highly aggressive NRAS mutated cutaneous melanoma. We present the clinical course, unique morphology, angiogenic properties, growth characteristics and 3D cell culture, and results of the exome gene sequencing of an intensively pigmented melanogenic cell line MUG-Mel2. Amongst several genetic alterations, mutations in GRIN2A, CREBP, PIK3C2G, ATM, and ATR were present. These mutations, known to reinforce DNA repair problems in melanoma, might serve as potential treatment targets. The aggressive behavior and the obtained phenotype in 3D culture reveal a perfect model for research in the field of NRAS mutated melanoma.

IV-393

Synthesis of evidence in laboratory animal research

Marjolein Schilders-van Boxel, Pieter Roelfsema, Herman Koëter, Jan-Bas Prins, Henriëtte Bout, Coenraad Hendriksen, Wim de Leeuw, Reineke Hameleers, Frauke Ohl †, Frank Dales and Leane van Weereld

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands

Marjolein.vanboxel@rvo.nl

In this position statement, NCad describes the use of various Synthesis of Evidence (SoE) methods in planning and conducting laboratory animal research and the contribution of these methods to 3Rs policy. According to NCad, SoE makes a crucial contribution to the quality of laboratory animal research, yet the NCad also points out that SoE can sometimes lead to an increase in the number of laboratory animals in an experiment because this improves the study design in certain situations. An analysis of many animal procedure publications does reveal, however, that there is often a lack of essential information relating to the experimental design.

SoE is considered an umbrella term for the various forms of classifying and evaluating available scientific knowledge as substantiation for a proposed animal or other procedure. Within these various forms, narrative reviews (descriptive literature reviews) are the most flexible and systematic reviews (SRs) are the most complete and time-intensive. In addition, open-access databases can be consulted with regard to the choice of animal models or 3R alternatives. Expert panels can also be used to discuss a specific scientific question.

NCad regards SoE in all its facets essential to enhance the quality of research questions and the design of laboratory animal research. The exact chosen SoE form depends on the specific research question and available knowledge. Limitations to the application of SoE are that essential information regarding the design of the procedure often lack from publications, negative results are seldom published and the results of animal procedures can not be disclosed due to professional confidentiality.

To encourage the application of SoE, NCad makes a number of recommendations to the field of biomedical research: 1) Encourage the application of a documented SoE in the design of a research project that considers the use of laboratory animals, but bear in mind that the scope and depth of the SoE depends on the available knowledge regarding the research question and the field. 2) When providing grants, the providers of those grants should promote the full spectrum of SoE, in particular also the creation and updating of relevant databases regarding the applicability of animal models. 3) Assessors of projects involving animal procedures, such as the Animal Ethics Committees (DECs), the Central Authority for Scientific Procedures on Animals (CCD), and Animal Welfare Bodies (IvDs) are advised to critically assess the application of SoEs during the assessment of projects. 4) Encourage attention and appreciation amongst researchers for the importance of publishing negative results and replications of earlier studies by, for example, incorporating this subject more firmly in training courses and in the criteria by which scientists are assessed.

NCad appointed an ad hoc expert working group at European level, comprising members from the Netherlands and other Member States, to draw up a harmonised code of practice for applying the SoE concept to the process of choosing between an animal procedure, an alternative procedure or abandoning an experiment. That code will also include issues with regard to responsible animal use.

This poster will give an overview of the advisory report by the NCad and the actions taken by the expert working group ever since.

Reference

https://english.ncadierproevenbeleid.nl/advice/documents/publications/16/7/19/soe

IV-688

Using *in vitro* data to compare endocrine disruption potential in hazardous chemicals currently found in children's products and their proposed alternatives

Marissa N. Smith[1], Rachel M. Shaffer[1], Elaine Cohen Hubal[2] and Elaine M. Faustman[1]

[1]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States; [2]Chemical Safety for Sustainability Research Program, US Environmental Protection Agency, Research Triangle Park, NC, United States

rissa8@u.washington.edu

Safer chemical substitutions are often identified by alternatives assessments. This project examines how *in vitro* data can fill gaps in alternatives assessment for hazardous chemicals found in children's consumer products by comparing European Chemical Agency's (ECHA) Endocrine Disruptor Substances of Concern classification with the toxicological prioritization index (ToxPi) score calculated based on the United Stated Environmental Protection Agency's ToxCast Database. The ECHA Classification is primarily derived from human and animal studies, and the ToxPi Score is based on *in vitro* assay results. Three chemical groups were considered: phthalates, parabens and bisphenols. Though alternative chemicals were rarely classified as endocrine disruptors by the ECHA, the *in vitro* ToxPi scores for alternative chemicals were slightly higher for bisphenols and phthalates. The results from this case study suggest that *in vitro* data can help fill gaps when existing classifications are incomplete

Theme V – Systems Biology and Big Data

Coordinators
Catherine Mahony, Procter & Gamble, Bagshot, Surrey, United Kingdom
Tom Knudsen, National Center for Computational Toxicology, US EPA, Research Triangle Park, NC, United States

Oral Presentations

Session V-1: Harnessing Big Data for Decision Making at Different Levels

Co-Chairs
Thomas Knudsen, US EPA, Research Triangle Park, NC, United States
Gladys Ouédraogo, L'Oréal R&I, Aulnay sous Bois, France

V-1-342

Use of transcriptional profiling in *in vitro* systems to determine the biological activity of chemicals of interest

Jorge Naciff, Yuquing Shan, Xiaohong Wang, Raghunandan Kainkaryam, Xingtao Wei, Lori Reinsalu, Renee Schinaman, Nadira De Abrew and George Daston
The Procter and Gamble Company, Cincinnati, OH, United States
naciff.jm@pg.com

The development of *in vitro* assays to assess chemical safety, as alternatives to animal testing, has become an important undertaking in toxicology research. Moreover, the research in this field has been moving more into the definition of chemical's mechanism of action, with clear identification of key events leading to adverse health effects. The ultimate goal is to rely less in apical endpoints, derived from *in vivo* studies, for the assessment of chemicals' safety. Thus, the need is not only for *in vitro* predictive toxicological assays, but also for these assays to provide relevant information of the biological activity associated with chemicals of interest, and with that, a better understanding of the underlying mechanisms of potential toxicity. We have focused our research in the use of transcriptional profiling in *in vitro* systems, a small number of cultured cell types enriched in relevant pathways for various modes of action, to determine the biological activity of chemicals of interest. Our data supports the hypothesis that transcriptional profiling of chemical-treated cultured cells, in a time and dose-dependent manner, provides data to characterize chemicals of interest based on their biological activity. This information can lead to a better understanding of key events underlying mechanisms of potential toxicity.

V-1-659

A model for estimating systemic toxicity points-of-departure using chemical, biological, kinetic and study covariates

Gladys Ouédraogo[1], Matthew Martin[2,3], LyLy Pham[2], Jacques Clouzeau[4], Sophie Loisel-Joubert[4], Delphine Blanchet[4], Hicham Noçairi[1], Woodrow Setzer[2], Richard Judson[2], Chris Grulke[2], Kamel Mansouri[2] and Lisa Truong[5]
[1]L'Oréal R&I, Aulnay sous Bois, France; [2]NCCT-ORD-EPA, Research Triangle Park, NC, United States; [3]Pfizer, Groton, CT, United States; [4]L'Oréal Safety R&I, Worldwide Safety Evaluation, Paris, France; [5]Oregon State University, Corvallis, OR, United States
gouedraogo@rd.loreal.com

Since 2013, repeated-dose systemic toxicity for cosmetics can no longer be assessed utilizing animal testing. This project aims to advance predictive approaches in this field by leveraging different data types – cheminformatics, bioactivity, kinetic parameters, legacy study covariates – to predict systemic toxicity points-of-departure (POD). A cross-validated and externally-tested random forest model was built using 1201 chemicals to derive estimates of study-level POD. Baseline performance was set with the study covariates alone – ~20% of the POD variance –, while adding the mean POD for each chemical explained ~70% of the variance; giving a performance benchmark. The model built using all features explained 38% of the variance in the external test set compared to 20-30% with isolated features. The model output provides a reliable estimate of POD with uncertainty quantified. This can be used with other data in a weight of evidence approach when evaluating repeated dose systemic toxicity.

V-1-635

Human brain model for analysis of pathways (Brain MAPS)

Gavin T. Knight[1,2], Maria Estevez-Silva[1,2], Deborah Chasman[3,4], Ethan Lippmann[1,2], Lydia M. T. Ashton[5], William Sethares[6], Rebecca Willett[6,7], Sushmita Roy[3,4] and Randolph S. Ashton[1,2]

[1]Biomedical Engineering, University of Wisconsin, Madison, WI, United States; [2]BioNATES, Wisconsin Institute for Discovery, Madison, WI, United States; [3]Computer Science, University of Wisconsin, Madison, WI, United States; [4]Systems Biology, Wisconsin Institute for Discovery, Madison, WI, United States; [5]Economics, University of Wisconsin, Madison, WI, United States; [6]Electrical and Computer Engineering, University of Wisconsin, Madison, WI, United States; [7]Optimization, Wisconsin Institute for Discovery, Madison, WI, United States

gavintknight@gmail.com

Brain MAPs is envisioned as a novel high throughput screening platform to recapitulate critical aspects of human central nervous system (CNS) development *in vitro* for neurotoxicological studies. Specifically, it is designed to recapitulate the cell phenotype diversity and tissue cytoarchitecture characteristic of the developing human CNS while maintaining the requisite sensitivity to detect cell phenotype-specific toxicity and identify the correlated signaling pathways disrupted by drug/chemical exposure. This is achieved using engineered, chemically-defined culture systems, diverse high-throughput measurements including imaging and omics profiling, and modeling these data to reproducibly instruct *in vitro* morphogenesis of CNS tissues from human neural stem cells (hNSCs). The hNSCs have been patterned to direct biomimetic tissue growth and differentiated to cell types found throughout the human embryo's rostrocaudal and dorsoventral neuraxis. Thus, Brain MAPs enables development of a pipeline for phenotype-specific, quantitative high-throughput developmental neurotoxicity studies.

V-1-409

Mechanistic modeling of developmental defects through computational embryology

Thomas Knudsen[1], Sid Hunter[2], Nancy Baker[3], Richard Spencer[3], James Glazier[4], George Daston[5] and Aldert Piersma[6]

[1]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC, United States; [2]NHEERL, US EPA, Research Triangle Park, NC, United States; [3]Leidos, Research Triangle Park, NC, United States; [4]Indiana University, Bloomington, IN, United States; [5]Procter & Gamble Co., Cincinnati, OH, United States; [6]National Institute for Public Health and the Environment RIVM, Bilthoven, The Netherlands

knudsen.thomas@epa.gov

An important consideration for the 3Rs is to identify developmental hazards of drugs and environmental chemicals utilizing mechanism-based *in vitro* assays (e.g., ToxCast) and *in silico* predictive models. Steady progress has been made with multicellular agent-based models (ABMs) that recapitulate morphogenetic drivers for angiogenesis, somitogenesis, urethrogenesis, and palatogenesis. Next up are ABMs for the neurovascular unit, endocardial cushions, and neural tube closure. These models offer a heuristic approach to reconstruct tissue dynamics from the bottom-up, cell-by-cell and interaction-by-interaction. Individually, they simulate emergent phenotypes and can be used to predict adverse outcomes or "cybermorphs" that bring an AOP to life through multicellular computational and spatial dynamics. Collectively, their compilation into an integrated "virtual embryo" motivates the construction of novel ontology systems that integrate molecular pathways, cellular behaviors, and *in vitro* data on chemical-biological interactions with extant knowledge of embryology.

This abstract does not reflect US EPA policy.

Session V-2: Future of Big Data in 3Rs and Recommendations – Round Table

Chair
Catherine Mahoney, Procter & Gamble, Bagshot, Surrey, United Kingdom

V-2-494

Future of big data in 3Rs and recommendations

Catherine Mahony
Procter & Gamble, Bagshot, Surrey, United Kingdom
mahony.c@pg.com

This roundtable session will explore what big data means for the 3Rs and how a deeper understanding of biology might play a role in eventually redefining decision making. Panellists will review the progress being made in big data for the 3Rs, attempt to lay out key challenges and engage in discussion on what is needed to further its practical application, both now and in the future. To harness big data and create meaning from it the infrastructure and resources for storage and analysis need to be up to par, we need to be able to extract relevant data and mine it for research purposes and as with any other research effort having a clear hypothesis, showing methodology and describing confidence in results is paramount.

Session V-3: Best Practices for Modeling Data

Co-Chairs
Raymond R. Tice, National Institute of Environmental Health Sciences, Durham, NC, United States
Glenn Myatt, Leadscope Inc., Columbus, OH, United States

V-3-710

Developing systems models for safety decision making: Challenges for improving confidence

Andrew White, Yeyejide Adeleye, Jin Li, Cameron Mackay and Alistair Middleton

Safety Environmental Assurance Centre, Unilever, Bedford, United Kingdom
andrew.white@unilever.com

Growth in the available data within the biological and toxicological community is increasing the range of tools available to integrate this information to aid chemical risk assessment in the development of non-animal approaches. However, transforming available data sets and tools for risk assessment highlights challenges around transparency, applicability, knowledge and estimation of uncertainty. Together these emphasise the need for best practice, analogous to those already undertaken in other fields, to increase confidence in the application and ultimately the regulatory submission of mathematical and computational models.

We provide examples in the development of statistical, mechanistic and quantitative systems models on how the approximation of the underlying biological system could be defined as fit for purpose. We further show how these disparate sources of information can inform on emergent properties to rationalise dose metrics such as tipping points between adaptive and adverse effects useful to risk assessment decision.

V-3-647

The development of *in silico* toxicology protocols

Glenn Myatt, Dave Bower, Kevin Cross, Catrin Hasselgren, Scott Miller and Donald Quigley

Leadscope, Inc., Columbus, OH, United States
gmyatt@leadscope.com

This project is generating a series of protocols to support the prediction of a series of major toxicological endpoints (e.g., genetic toxicity, carcinogenicity, acute toxicity, reproductive and development toxicity). These protocols are being developed through an international cross-industry consortium to reflect the state-of-the art in computational toxicology for hazard identification and characterization. The consortium is led by Leadscope and includes 45 organizations from international regulatory agencies and government research laboratories in the US, Canada, Japan, and Europe as well as large companies from the various industrial sectors (e.g., pharmaceutical, food, cosmetics, agrochemicals), academic groups and other stakeholders. The protocols will ensure any *in silico* toxicological assessments are performed in a consistent, repeatable, well-documented and defendable manner. This includes how to assess the reliability and relevance of data/predictions, how an expert review of the results may be performed and how an overall assessment may be performed based on the weight-of-the-evidence.

V-3-408

Modeling the role of microglia during neurovascular development

Katerine Saili[1], Aymeric Silvin[2], Todd Zurlinden[1], Andrew Schwab[3], Sidney Hunter[3], Nancy Baker[4], Florent Ginhoux[2] and Thomas Knudsen[1]

[1]ORD/NCCT, US EPA, Research Triangle Park, NC, United States; [2]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore; [3]ORD/NHEERL, US EPA, Research Triangle Park, NC, United States, [4]Leidos, Research Triangle Park, NC, United States
saili.katerine@epa.gov

Microglia, resident brain macrophages, have important roles in blood-brain barrier (BBB) development and during focal BBB disruption. We reconstructed these complex dynamics utilizing computational and molecular methods: 1) constructing a systems map of BBB development from known signaling events among cells of the neurovascular unit (NVU), including assay target genes from the ToxCast database; 2) building an agent-based model to recapitulate the reciprocity of microglial (CSF1) x endothelial (VEGF) signals and predict the quantitative impairment of microvascularization during CSF1R inhibition; and 3) testing this prediction utilizing a mouse model wherein embryonic microglia were ablated by anti-CSF1R antibody, and an organotypic culture model of the human NVU on a chip. These complementary models provide powerful tools for testing the potential roles of microglia in developmental NVU toxicity.

This abstract does not reflect US EPA policy.

V-3-694

Open analytical challenges to crowdsource biomedical research

Solveig Sieberts and Lara Mangravite

Sage Bionetworks, Seattle, WA, United States
solly.sieberts@sagebase.org

Explosive growth in high-dimensional biomedical data generation has led to the need for rapid development and benchmarking of advanced computational methods to produce actionable knowledge from these datasets. A popular approach to benchmark such methods and understand the capacity for scientific insight from a dataset is the organization of unbiased crowdsourcing-based science competitions/challenges. DREAM engages diverse communities of computational experts to leverage the "*wisdom of crowds*" to solve specific biomedical problems. DREAM organizers have launched over 40 successful challenges, which have attracted over 8,000 participants and resulted in over 100 publications addressing pressing computational issues and biomedical problems. DREAM challenges, and related efforts, provide a promising opportunity to effectively assimilate the rich knowledge embedded within the research community and establish community consensus in research outcomes.

Session V-4: Resources and Tools for State of the Art Systems Modeling

Co-Chairs
Roland Grafstrom, Karolinska Institute, Stockholm, Sweden
Hiroaki Kitano, Systems Biology Institute, Tokyo, Japan

V-4-525

In vitro-based high-throughput/ high-content screening and omics-driven informatics support integration of systems biology and big data in alternative testing methods

Roland Grafstrom[1], Penny Nymark[1], Vesa Hongisto[2] and Pekka Kohonen[1]

[1]Karolinska Institute, Stockholm, Sweden; [2]Toxicology Department, Misvik Biology, Turku, Finland

roland.grafstrom@ki.se

The rapidly developing "Big Data era" in toxicology involves generation of large amounts of safety testing data and the need to interpret the results relative steadily increasing amounts of related information in databases. Cell culture models serve for high-throughput screening analysis, allowing libraries of drugs, chemicals and nanomaterials to be effectively assessed and ranked for cytotoxicological properties. In parallel, microscopy-driven high-content analyses of morphology and immunochemical markers, and genomic profiling data opens for mechanistic cytopathological interpretation. Bioinformatics tools additionally permit overview and visualization of new results relative existing relevant data. Ideally, the combined use of the above technologies allows for coupling to adverse outcome pathway descriptions. Tiered workflows combine these methods into safety evaluations building on systems toxicology. Applications and challenges related to the above concepts will be discussed.

References

Grafström, R. C. et al. (2015). Toward the replacement of animal experiments through the bioinformatics-driven analysis of 'omics' data from human cell cultures. *Altern Lab Anim 43*, 325-332.

Kohonen, P. et al. (2014). Cancer biology, toxicology and alternative methods development go hand-in-hand. *Basic Clin Pharmacol Toxicol 115*, 50-58.

Kohonen, P. et al. (2017). A transcriptomics data-driven gene space accurately predicts liver cytopathology and drug-induced liver injury. *Nat Commun 8*, 15932.

V-4-207

A new tool for aligning assay endpoints to adverse outcome pathways

Shannon Bell[1], Lyle Burgoon[2], Jason Phillips[3], Dave Allen[1], Warren Casey[4] and Nicole Kleinstreuer[4]

[1]ILS, Morrisville, NC, United States; [2]US Army ERDC, Research Triangle Park, NC, United States; [3]Sciome, Research Triangle Park, NC, United States; [4]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, United States

Shannon.Bell2@nih.gov

A critical toxicological challenge is linking endpoints measured in non-animal approaches to adverse physiological responses *in vivo*. The adverse outcome pathway (AOP) framework allows placement of these molecular, cellular, and tissue-level endpoints into a biologically relevant context. The National Toxicology Program's Integrated Chemical Environment (ICE) web resource houses curated data from *in vivo*, *in vitro*, and *in silico* endpoints. We present a new feature of ICE that maps assay endpoints to key events within AOPs. We demonstrate how this feature can be used to identify data gaps, build confidence in mechanistic plausibility and relevance, and provide insights on potential adverse outcomes using AOPXplorer. This presentation will use the skin sensitization AOP and putative AOPs for androgen and estrogen receptor pathways to demonstrate the utility of this feature.

This was funded with U.S. federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

V-4-246

Development of a repeated dose toxicity mode of action-based ontology

Bertrand Desprez[1], Barbara Birk[2], Bas Blaauboer[3], Alan Boobis[4], Paul Carmichael[5], Mark Cronin[6], Richard Currie[7], George Daston[8], Bruno Hubesch[9], Paul Jennings[10], Martina Klaric[11], Dinant Kroese[12], Catherine Mahony[13], Gladys Ouédraogo[14], Aldert Piersma[15], Andrea Richarz[16], Michael Schwarz[17], Jan Van Benthem[15], Bob Van de Water[18] and Mathieu Vinken[19]

[1]Cosmetics Europe, Brussels, Belgium; [2]BASF, Ludwigshafen, Germany; [3]University of Utrecht, Utrecht, The Netherlands; [4]Imperial College, London, United Kingdom; [5]Unilever, Bedford, United Kingdom; [6]Liverpool Johns Moore University, Liverpool, United Kingdom; [7]Syngenta, Alderley Park, United Kingdom; [8]Procter & Gamble, Cincinnati, OH, United States; [9]CeficBel, Brussels, Belgium; [10]Medical University of Innsbruck, Innsbruck, Germany; [11]Cosmetics Europe, Brussels, Belgium; [12]TNO, The Hague, The Netherlands; [13]Procter and Gamble, Bagshot, Surrey, United Kingdom; [14]L'Oréal, Aulnay sous Bois, France; [15]National Institute for Public Health and the Environment RIVM, Bilthoven, The Netherlands; [16]European Commission Joint Research Centre, Directorate for Health, Consumers and Reference Materials, Ispra, Italy; [17]University of Tübingen, Tübingen, Germany; [18]Universiteit Leiden, Leiden, The Netherlands; [19]Vrije Universiteit Brussel, Brussels, Belgium
bdesprez@cosmeticseurope.eu

Recent research programmes in toxicology and risk assessment showed the critical importance of understanding modes of action of chemicals to enable predictions of human health effects. High-throughput technology generates a considerable amount of data. It triggers the need for a structured system, an ontology, to support toxicity predictions. Developing a repeated dose toxicity ontology (RDTO) requires addressing four main pillars: chemistry; kinetics & exposure; mode of action and toxicological effects. By integrating multiple adverse outcome pathways (AOPs) and their links, the RDTO produces an AOP-network. It reflects a realistic *in vivo*-like toxicological exposure-response scenario, captures homeostatic adaptations of biological systems, defines critical key events (KEs) and quantify relevant key event relationships (KERs). The RDTO waives *in vivo* testing while enabling human-based *in vitro* experimentation, and supports the use of AOPs in contemporary risk assessment.

V-4-711

Computational modeling: Moving from data mining to understanding systems

Elan Ohayon[1] and Ann Lam[2]

[1]Green Neuroscience Laboratory, Neurolinx Research Institute, La Jolla, CA, United States; [2]Physicians Committee for Responsible Medicine, Washington, DC, United States
ohayon@greenneuro.org

Increased computational processing speeds and the availability of seemingly limitless data storage have brought about the age of "big data" and "deep learning" which have become almost synonymous with "computational approaches". However, rather than replace animal research these trends have often led to worsening scientific, clinical and ethical outcomes. Lost in all this, is the power of certain forms of theoretical computational modeling. These alternate methods allow for rapid investigation using abstractions and evolution. Here we present a study of neurodegeneration using spatial neural networks that may have broad applicability to aging and age-related conditions such as dementia. We demonstrate how these simulations may help explain behaviors seen in systems ranging from stem cell cultures to human neural activity. In doing so, we underscore the power of such approaches to go beyond brute force data mining and help researchers gain deeper understanding without the use of animals.

Session V: Systems Biology and Big Data

Poster Presentations

V-371

Indicators, management and utilisation of data for monitoring laboratory animal use and 3R alternatives

Marjolein Schilders-van Boxel, Coenraad Hendriksen, Herman Koëter, Jan-Bas Prins, Henriëtte Bout, Wim de Leeuw, Pieter Roelfsema, Frauke Ohl † and Frank Dales

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands

Marjolein.vanboxel@rvo.nl

In March 2015, the Minister of Agriculture (EZ) asked the National Committee for the protection of animals used for scientific purposes (NCad) to issue an advisory report on the management and use of data for monitoring laboratory animal use and 3R alternatives for the Reduction, Replacement and Refinement of laboratory animal use. The guiding principle of the advisory report, which was published in two parts, is the Minister's ambition to promote animal welfare and to minimise the number of animal procedures as much as possible.

The NCad recommends compliance with government policy on open data, with regard to all information made available by the government on laboratory animal use, animal procedures, and 3R alternatives. Publication of such material is subject to the restrictions imposed by privacy sensitivity and the protection of intellectual property. In this regard, Dutch practice goes further than is usual in Europe. In the interests of establishing a level playing field, The NCad advises the Minister to commit herself to a European open data policy as well. The open data should be made available in the form of a central data warehouse that is publicly accessible via a website. This central data warehouse should also contain information provided by practitioners working in the field. The data sets contained in this data warehouse should be structured in a way that enables matrix connections to be established, and that enables data to be regrouped and analysed. Furthermore it must be ensured that links can be established with information about laboratory animal use held in the existing databases. This must be done in a way that will make it possible to generate regular, detailed trend analyses of laboratory animal use in prioritised categories of research.

The improved insight into laboratory animal use that this will deliver, together with the development and application of 3R alternatives, may result in the more efficient use of this information. It may also improve policy management and study design, while providing a better basis for the development and implementation of 3R alternatives.

The centralised data warehouse should be built up in stages, whereby the government should take the initiative during the first stage.

The minister of Agriculture has embraced the opinion of the NCad and has commissioned the National Institute for Public Health (RIVM) to set up the centralised data warehouse. This poster will give an overview of the advisory report by the NCad and the actions taken by the stakeholders ever since.

Reference

https://www.ncadierproevenbeleid.nl/documenten/rapport/2015/11/26/ncad-advisory-report-data-and-monitoring

V-378

The effects of the Candidate Chemical List 4 (CCL4) on differentiation and cytotoxicity in mouse embryonic stem cells

Harriette Nichols, Susan Jeffay, Maria Hoopes and Edward Hunter

Integrated Systems Toxicology Division, US Environmental Protection Agency, Research Triangle Park, NC, United States

nichols.harriette@epa.gov

The potential for environmental chemicals to produce birth defects is largely unknown. Mouse embryonic stem cells were used to profile the bioactivity of chemicals on the EPA Contaminant Candidate List 4 (CCL4). Differentiation and cytotoxicity were evaluated for 81 non-volatile chemicals on culture days 4 (gastrulation stage) and 9 (cardiomyocyte stage). Stage specific protein markers were used to assess differentiation. Twenty one chemicals were found to have bioactivity on culture day 4, 26 chemicals on day 9, and 20 chemicals had activity on both days. Based on previous work which showed that the combination of cell number on culture day 4 and differentiation on day 9 best correlate to *in vivo* toxicity. Our analysis suggests that, at ≤ 100uM, 24 CCL4 chemicals would be categorized as potential developmental toxicants. These studies provide a basis to prioritize CCL4 chemicals for in-depth assessment.

This abstract does not represent EPA policy.

V-422

An investigation of the antiandrogenic effects of propyl paraben on male reproductive system

Nurhayat Barlas and Ecem Özdemir
Hacettepe University, Ankara, Turkey
barlas@hacettepe.edu.tr

We investigated possible antiandrogenic profiles of propyl paraben following doses at 10, 250 and 750 mg/kg/day to castrated immature male Wistar rats. According to Hershberger Bioassay, at their 6 weeks of age, rats were castrated and given 8 days for recovery. Then, rats were divided into six groups including the vehicle control, negative control (0.4 mg/kg/day TP), positive control (3 mg/kg/day FLU + TP) and propyl paraben treatment groups (10, 250, 750 mg/kg/day PP + TP). Also, body weights and food and water consumption were noted daily. After 10 days of treatment period, rats were killed and the accessory sex organs, liver and kidneys were weighed. Testosterone propionate, increased the weights of accessory sex glands, liver and kidney at 0.4 mg/kg/day and flutamide, decreased all the TP-stimulated organ weights at 3.0 mg/kg/day. Propyl paraben significantly decreased the all organ weights at each dose of 250 and 750 mg/kg/day. Thus, we found that propyl paraben is antiandrogenic within the supported results of increasing LH levels and histopathologic results such as atrophy, decrease in epithelium height and hyalinization on androgenic tissues.

V-492

Gene expression profiling-based characterization and optimization of a 3D alveolar tetra-culture *in vitro* model

Diego Marescotti[1], Tommaso Serchi[2], Yang Xiang[1], Karine Baumer[1], Remi Dulize[1], Dariusz Peric[1], David Bornard[1], Emmanuel Guedj[1], Alain Sewer[1], Karsta Luettich[1], Sebastien Cambier[2], Servane Contal[2], Elisa Moschini[2], Marja Tallika[1], Florian Martin[1], Arno C. Gutleb[2], Nikolai V. Ivanov[1], Stefan Frentzel[1], Manuel C. Peitsch[1] and Julia Hoeng[1]
[1]Philip Morris International, Neuchâtel, Switzerland; [2]Luxembourg Institute of Science and Technology, Esch-sur-Alzette, Luxembourg
diego.marescotti@pmi.com

In order to further optimize an existing *in vitro* model for inhalation toxicity, a tetraculture system (Klein et al., 2013) which combines lung epithelial cells with endothelial cells, macrophages and mast cells has been analysed molecularly. The study aimed at exploring gene-expression profiles to characterise the dynamics elicited by each cell type assembled into the final tetraculture system. Taking advantage of Network Perturbation Amplitude (NPA) scoring methods macrophages appeared to make the biggest contribution to the overall network perturbations, promoting high basal levels of oxidative stress and inflammation. Endothelial cells and mast cells overall had much less effect on the investigated NPA scores. These observations enabled optimization of the tetraculture model, using rested macrophages, which decreased the basal inflammatory and cell stress status of the culture.

Reference

Klein, S. G., Serchi, T., Hoffmann, L. et al. (2013). An improved 3D tetraculture system mimicking the cellular organisation at the alveolar barrier to study the potential toxic effects of particles on the lung. *Part Fibre Toxicol 26*, 31. doi:10.1186/1743-8977-10-31

V-613

Development of new data analysis method KY-method

Kohtaro Yuta
In Silico Data, Ltd., Chiba, Japan
k-yuta@insilicodata.com

Introduction: The KY-method is a data analysis method featuring multistep re-sampling process. Currently 6 types of KY-method (U.S. Patent No. 7725413, 2010) are developed. There are three types as classification methods of two classes, and three types as multiple regression methods. The KY method achieves an extremely high classification rate in the two-class classification method and realizes an extremely high determination coefficient in the multiple regression method.

Basic composition of KY-method: In the KY-method, various existing data analysis methods are used as classification method and multiple regression method. Therefore, the KY-method is not a specific data analysis method but a "*meta-analysis method*" developed in combination with an existing data analysis method. For example, in the two-class classification KY-method, various data analysis methods such as SVM (Support Vector Machine), AdaBoost, NN (Neural Network), Bayesian Analysis and other various discriminant analysis methods are applied. Likewise, in the multiple regression KY-method, it is possible to apply various methods such as linear/nonlinear regression, logistic regression, PLS (Partial Least Squares), etc. in the multiple regression method.

Excellent feature of the KY-method: The KY-method does not deteriorate the accuracy of data analysis even if a large number of samples are used. Therefore, the KY-method has a powerful and optimum function corresponding to the future big data era. (1) Sample number free: The KY method has a feature that data analysis accuracy does not drop even if the number of samples is extremely large. This is because sample groups are classified into small groups at individual step, so that the number of samples is relatively small, which makes it possible to avoid degradation of data analysis accuracy. (2) By the KY-method, classification accuracy and coefficient of determination can be increased: In the KY-method, noise samples are put together and transfer to the next step. For this reason, it is possible to analyze the data without noise samples within each step. As a result, data analysis can be performed without decreasing the classification rate and the determination coefficient. (3) The KY-method is a "meta-analysis method" to which existing methods are applied as a data analysis method applied at individual stages. This makes it easy to incorporate data analysis methods to be developed in the future, and can always work with the latest methods.

Types of KY-method: The KY-method currently has three types as two class classification methods and also three types as multiple regression method are developed. Two class classification KY-method: (a) Two model KY-method, (b) One model KY-method, (c) Model free KY-method; Multiple regression KY-method: (a) KY-Fitting with DA (Discriminant analysis), (b) KY-Fitting with no DA (Discriminant analysis), (c) Model free KY-fitting.

Combination of the KY-method and other methods: The method that the KY-method can apply at the present time is a two-class classification method and a multiple regression method. The KY-method can be executed not only independently but also in cooperation with data analysis methods with different analysis objectives. For example, in PCA using a very large number of samples, since the overlapping of samples is large and the separation rate is low, it is practically impossible to analyze using a biplot diagram. In the combination analysis of KY-method and PCA, by obtaining the principal component map for each step of the KY method, a separation rate is high and a diagram which facilitates data analysis is obtained.

Summary: The KY-method is a "*meta-analysis method*" that can improve the data analysis power possessed by the original methods. In the poster, more details on the KY-method will be discussed.

V-640

Using product intake fraction to characterize human exposure to chemicals in consumer products for green chemistry

Olivier Jolliet[1], Peter Fantke[2] and Lei Huang[3]

[1]University of Michigan, School of Public Health, Environmental Health Sciences, Ann Arbor, MI, United States; [2]Technical University Denmark, Lyngby, Denmark; [3]Environmental Health Sciences, University of Michigan, School of Public Health, Ann Arbor, MI, United States

ojolliet@umich.edu

To consistently and quantitatively compare human exposure to chemicals in consumer products in the context of green chemistry, we introduce the concept of product intake fraction (PiF), as the fraction of a chemical within a product that is eventually taken in by the human population. This metric enables consistent comparison of exposures during consumer product use for different product-chemical combinations, exposure duration, exposure routes and pathways and for other life cycle stages. It can then be combined with High Throughput Toxicity measures to screen risks associated with chemicals in products. This presentation will demonstrate how the product intake fraction can be determined for chemicals in personal care products (800+ chemicals), building materials (200+ chemicals) and packaging materials, using parsimonious models adapted for high throughput exposure assessment. The PiF ranges over several orders of magnitude depending on mass balance, removal processes and chemical properties, demonstrating its utility within life cycle assessment, risk assessment, alternatives assessment and green chemistry contexts.

Theme VI – 3Rs in Academia

Coordinators
Silvya Stuchi Maria-Engler, University of São Paulo, São Paulo, Brazil
Mardas Daneshian, CAAT-Europe, University of Konstanz, Konstanz, Germany

Oral Presentations

Session VI-1: Evaluation of the Global Impact of the 3Rs in Education and Training

Co-Chairs
Nick Jukes, InterNICHE, Leicester, United Kingdom
Silvya Stuchi Maria-Engler, University of São Paulo, São Paulo, Brazil

VI-1-517

Alternative tools and approaches for replacement in veterinary education and training

Nick Jukes[1], Olivier Berreville[2] and Siri Martinsen[3]
[1]InterNICHE, Leicester, United Kingdom; [2]InterNICHE Canada, Halifax, Canada; [3]NOAH and InterNICHE Norway, Oslo, Norway
coordinator@interniche.org

According to the InterNICHE Policy, replacement alternatives in veterinary medical education and training comprise non-animal tools and humane interactions with animals. Non-animal tools include software, virtual reality, models, mannekins and other training devices. Alternative approaches that involve animals include the use of ethically sourced animal cadavers, plastination and other preservation methods, and clinical learning opportunities with animal patients. Together with non-animal tools, they can replace animal experimentation, dissection of purpose-killed animals, and other instrumental animal use. This paper reviews some of the tools and approaches developed by teachers and companies for knowledge and skills acquisition in anatomy, physiology, pharmacology, surgery and other disciplines. It demonstrates the potential for full replacement of harmful animal use by providing case studies from veterinary faculties across the world, with particular emphasis on Canada and the US.

VI-1-560

MGDC model of sensitization about non-animal methods in education, research and testing at the national level

Mohammad Akbarsha
Bharathidasan University, Tiruchirappalli, India
akbarbdu@gmail.com

India has made rapid strides in the movement of "alternatives to animal use". The turning point has been establishment of a center for alternatives in the University system, the Mahatma Gandhi-Doerenkamp Center (MGDC), by the DZF. The NGOs of the country and MGDC got into a grand alliance. While the NGOs played their own cards, the MGDC targeted the end users - academic institutions, research organizations, industry and regulatory authorities, and dealt with each in the language they would understand. MGDC's approach included hands-on training, thus raising alternatives to the level of a science. Even within the educational scenario, stakeholders of each domain were tackled separately. Today, India is practically an animal-dissection free country, also with great changes in the scenario of animal use in experiments and testing. MGDC is by far the best to have achieved such a dramatic change in the scenario of animal use, and stands the best model to adopt.

VI-1-177

Social movements and legal imperatives for replacement in Serbia

Katarina Novaković
InterNICHE Serbia and ORCA Belgrade, Serbia, Belgrade, Yugoslavia
kahabg@gmail.com

Serbia's first Animal Protection Act came into force in 2009. Article 44 of the Act bans the harmful use of animals at primary, secondary and undergraduate levels. The Article was based on the InterNICHE Policy on Animals and Alternatives in Education and Training. This paper explores the chronology of events and factors which led to the adoption of the Act. From 2001 there were many political and social changes in the country. Students, professors and NGOs chose to think strategically in a number of fields. Many public debates on animal protection were held, with much media interest. Major technological advances made computer hardware and software affordable and very effective for education. Animal protection resolutions were adopted, the European Convention was ratified, and civil laws that guarantee conscientious objection were used. Reflections on the impact of the Act and of Article 44, including on Ethical Committees and on replacement using alternatives, will be given.

VI-1-214

Outreach, agreements and provision of alternatives: Facilitating replacement in the Ukraine and other former Soviet states

Dmitry Leporsky[1], Nick Jukes[2] and Corina Gericke[3]
[1]InterNICHE Ukraine, Kharkov, Ukraine; [2]InterNICHE, Leicester, United Kingdom; [3]Doctors Against Animal Experiments Germany, Cologne, Germany
m.dachnik@gmail.com

A major alternatives project from Ukrainian campaigners, InterNICHE and Doctors Against Animal Experiments (Germany) has achieved widespread replacement within Ukrainian life science education and training. Using the experience of InterNICHE in Russia, the work began in 2008 and is on-going. Strategies include outreach visits, demonstrations of alternatives, negotiations with teachers, formal agreements for replacement, and provision of computer hardware, software and training models. According to the signed agreements, over 50,000 animals (vertebrates and invertebrates) have been replaced in more than 50 departments. As the project is extended to include more former Soviet states including Belarus, Russia and others in Central Asia, further replacement is being achieved.

The 2015 Lush Prize for Training was awarded for this work.

VI-1-278

The end of harmful animal use in professional and higher education: An on-going process in Brazil

Róber Bachinski[1], Mariângela Freitas de Almeida Souza[2], Vanessa Bones[3], Rita de Cassia Maria Garcia[4], Rosangela Gebara[5], Valeska Regina Reque Ruiz[6], Luciano Alonso[7], Thales Trez[1,8], Simone Tostes Oliveira[4], Alexandro Aluisio Rocha[9], Gutemberg Alves[1,10], Rita Leal Paixão[1,11], Roseli Pizzigatti Klein[12], Débora Gasparetto[13], Rita Seabra[14], Nick Jukes[15] and Júlia Maria Matera[16]
[1]1R Institute of Promotion and Research for the Replacement of Animal Experimentation, Porto Alegre, Brazil; [2]National Forum for the Defense and Protection of Animals, Brazil; [3]Regional Council of Veterinary Medicine of Paraná (CRMV-PR), Curitiba, Brazil; [4]Veterinary Medicine Department, Federal University of Paraná, Curitiba, Brazil; [5]World Animal Protection, São Paulo, Brazil; [6]Faculty of Veterinary Medicine, Center of Higher Education of Campos Gerais, Campos Gerais, Brazil; [7]Laboratory of Anatomy, Federal Rural University of Rio de Janeiro, Rio de Janeiro, Brazil; [8]Institute of Science and Technology, Alfenas Federal University, Minas Gerais, Brazil; [9]Animal Science Department, Faculty of Agrarian Sciences, Federal University of Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil; [10]Institute of Biology, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil; [11]Department of Physiology and Pharmacology, Biomedical Institute, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil; [12]Piauiense Association for Protection and Love of Animals, Teresina, Brazil; [13]Department of Industrial Design, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil; [14]Fund for the Replacement of Animals in Medical Experiments (FRAME), Nottingham, United Kingdom; [15]International Network for Humane Education (InterNICHE), Leicester, United Kingdom; [16]Department of Surgery – Veterinary Medicine College and Zootecny, São Paulo University, São Paulo, Brazil
rober.bachinski@gmail.com

The Brazilian Network for Humane Education (RedEH) – an independent group of academics from 10 Brazilian states, and international collaborators – has been pushing for change in the field of humane education. In 2016, with the support of InterNICHE and 18 international professors and organizations, RedEH requested that the Brazilian National Council for the Control of Animal Experimentation (CONCEA) ban harmful animal use in professional and undergraduate education. This was the first formal request for a total replacement of harmful animal use in education in Brazil, and represents a historic event in the advancement of scientific education. RedEH is open to all researchers working in humane education and new teaching methods in the life and health sciences. We aim to provide a collaborative and encouraging environment, including not only researchers from the life sciences but also product design, virtual game development and science communication.

VI-1-427

Knowledge, attitudes and practices on use of live animals in training in Kenya

Dennis Makau, Maryanne Kagai and Dennis Bahati
Africa Network for Animal Welfare, Nairobi, Kenya
dennis@anaw.org

ANAW, in partnership with InterNICHE has since 2010 been carrying out sensitization efforts and seminars on alternatives to use of animals for training and education. Coupled to this effort ANAW played a critical role in the drafting and adoption of regulations governing use of lab animals in research in Kenya. One of the efforts in place by ANAW has been to support the adoption of alternatives for teaching purposes in science oriented institutions. Five science oriented institutions and subsequently relevant departments in each institution, were randomly selected. A survey was carried out to establish the knowledge, attitudes and practices towards use of animals; and awareness thereof, about alternatives available for use in teaching. The survey was carried out using a questionnaire administered to heads of staff and different department heads in the various schools and courses. In the 5 institutions surveyed, animals were used for teaching various units in zoology, animal science and veterinary medicine courses. In veterinary schools, most dogs and cats are used for surgery purposes while pharmacology and physiology are the courses that use animals the most. All species are used for physiology and pharmacology experiments. Most (60%) of the respondents said that they would consider introduction of alternatives, 80% of the respondents had used various alternatives at different instances; 60% of the respondents thought that introduction of alternatives would have an effect understanding of students; 60% of respondents quoted unavailability of alternatives as the major hindrance to introduction of alternatives while 20% feared that the alternatives would reduce the level of understanding and 20% were not sure if the same level of understanding would be maintained. Different animals were used for different subjects and courses as indicated above. The most commonly used animals in teaching institutions in Kenya are cats and dogs at 14%. In conclusion, most trainers and training institutions they represented were willing to use alternatives. However, serious challenges on availability and effect on quality of training provided largely contributed to the aversion in some of the training fields.

Session VI-2: Knowledge Sharing in Promoting 3Rs Advances

Co-Chairs
Marilyn Brown, Charles River Laboratories, Wilmington, MA, United States
Tracey Holley, European Commission Joint Research Centre, Ispra, Italy

VI-2-728

The North American 3Rs Collaborative – Facilitating opportunities to enhance the 3Rs in North America

Marilyn Brown
Animal Welfare and Training, Charles River, Wilmington, MA, United States
marilyn.brown@crl.com

The concepts of the 3Rs are found in regulation, guidance documents and institutional policies throughout North America (NA). Many individuals in the public and animal research believe there is more to be done. There is a need for the research community to better communicate, internally and externally, about 3Rs implementation strategies and advancement of the 3Rs. This communication would serve to share ideas and help avoid duplication of efforts (an increasing concern as resources become more problematic). Increased communication can also help identify opportunities for collaboration on activities aimed at more fully implementing the 3Rs across the field. The NC3Rs stands out as a model for achieving these goals but such an effort in the NA has been elusive. Major inclusive governmental or funding initiatives are lacking. This talk will discuss a broad based collaboration, including many areas of research (academia, industry, etc.) and many areas of expertise (veterinarians, research scientists, advocates, etc.) that have come together to address this opportunity.

VI-2-384

Facilitating better knowledge exchange to accelerate progress in the 3Rs

Tracey Holley
European Commission Joint Research Centre, Ispra, Italy
tracey.holley@ec.europa.eu

By mapping knowledge sources relevant to the 3Rs, examining how knowledge is being shared, and identifying possible gaps and opportunities, the European Commission's Joint Research Centre (JRC) has identified three main areas to be addressed in order to develop strategies to improve knowledge sharing to accelerate progress in the 3Rs. The JRC has a performed a review of the supply and demand status of 3Rs knowledge and created a detailed inventory of 800 knowledge sources relevant to the 3Rs. In parallel to this inventory, a public survey (of people working in the 3Rs area) was carried out which aimed to elicit individual input on what knowledge sources exist, how they are linked, and how they are currently being used to further the 3Rs.

Whilst there are many 3Rs relevant knowledge sources available, there is a need for better coordination and communication of the knowledge, as well as opportunities to enhance education and training. How this can be achieved is also explored here.

References

Directive 2010/63/EU of The European Parliament and of The Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J Eur Union L276*, 33-79.

Holley, T., Bowe, G., Campia, I. et al. (2016). Accelerating progress in the Replacement, Reduction and Refinement of animal testing through better knowledge sharing. EUR 28234 EN. Publications Office of the European Union. JRC103906

Russell, W. M. S. and Burch, R. L. (1959). *The Principles of Humane Experimental Technique*. South Mimms, Potters Bar, Herts, UK: Universities Federation for Animal Welfare.

VI-2-162

Attitudes towards the 3Rs in animal welfare bodies at eight Swedish universities

Elin Törnqvist[1,2] and Johan Lindsjö[1,3]
[1]Swetox, Södertälje, Sweden; [2]Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; [3]Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden
elin.tornqvist@swetox.se

The attitude towards the 3Rs was surveyed among members of Animal Welfare Bodies (AWB) at eight Swedish Universities to study the AWB compliance with the EU-directive 2010/63. A questionnaire with 34 quantitative closed-ended questions received responses from 45 of 90 members.

Comparable with previous surveys (NC3Rs, 2008; Nøhr et al., 2016), correct understanding of the 3R definitions was rather high (76-93%), but also aligned with some misconceptions for all three Rs. The 3Rs were not equally considered throughout the research process. For several questions, many respondents answered "I don't know", more often for areas covering Replacement/Reduction, indicating a stronger emphasis on Refinement. The overall attitude towards Refinement was positive. The tasks of the AWB, e.g. giving advice on the 3Rs and follow up on animal use in projects, informing about technical and scientific development within the 3Rs, and re-homing, was often not carried out in the AWB (11-42%), or "not known" (20-44%) by the respondents.

References

NC3Rs (2008). Views on the 3Rs. Survey Report 2008. National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, United Kingdom.

Nøhr, R., Lund, T. B. and Lassen, J. (2016). The Danish 3R survey: Knowledge, attitudes and experiences with the 3Rs among researchers involved in animal experiments in Denmark. Frederiksberg: Department of Food and Resource Economics, University of Copenhagen, Denmark (IFRO Report; No. 249).

VI-2-260

A common set of values and the 3Rs at Karolinska Institutet, Sweden

Viveka Hillegaart, Rafael Frias, Johannes Wilbertz and Brun Ulfhake

Comparative Medicine, Karolinska Institutet, Stockholm, Sweden
viveka.hillegaart@ki.se

Comparative Medicine (CM) was founded as an umbrella organization to develop a completely refurbished infrastructure for animal experiments at the Karolinska Institutet (KI). CM is built on vision that the 3Rs should permeate every aspect of care and use of laboratory animals at KI. The aim is to offer a cutting edge research environment set in a best practice and care culture fostered by the staff at CM. A thread of 3R activities based on evidence-based practice is being implemented and harmonization on the national level and within the EU is another goal. To accomplish these goals, CM has been placed directly under the Vice-Chancellor at KI. CM will have five laboratory facilities and a group of core facilities offering services to the research community consisting of 22 departments and several thousands of researchers.

To live up to regulations and regulatory requirements, CM has established a backbone of support for the laboratories and the animal facilities composed of an Infrastructure unit, an Education and training unit and an Executive office. To be able to implement the 3Rs concept from the Directive 2010/63/EU, CM has created a position as "Senior 3R officer" within the executive office and with the missions to make all the tree 3R visible both externally and internally in a transparent way, and that all research performed at KI shall be aligned with the 3R intentions of the Directive.

To accomplish this aspiration a program for the 3R:s have been set-up at KI. Highlights of this program are (1) a yearly event where employees researchers, students and others can exchange new concepts and results around animal welfare in biomedical research and the 3Rs, (2) custom tailored educational efforts in collaboration with the LAS education office to increase knowledge and awareness of the 3Rs, (3) to embed the animal welfare officer function (AWO) in the 3R office, and (4) the senior 3R officer is the chairperson of the local animal welfare body (AWB) at KI.

VI-2-280

Norecopa: A toolbox for the 3Rs in action

Adrian Smith[1], Karina Smith[1], Tim Allen[2], Øyvind Wærenskjold[3], Bente Bergersen[1], Siri Knudsen[1], Anton Krag[1] and Børge Fredriksen[1]

[1]Norecopa, Oslo, Norway; [2]AWIC, Beltsville, MD, United States; [3]Bitfarm, Kristiansand, Norway
adrian.smith@norecopa.no

The media are overflowing with material on animal care and use, much of which has not been specifically selected or peer-reviewed. The availability of so many resources makes it difficult to identify the best material for practising good science and the 3Rs. In addition, recent scientific reviews have revealed poor reproducibility of results from animal testing, indications of weak experimental design, and poor compliance with guidelines for reporting animal studies. If the situation is to improve, scientists need easy access to the best tools.

Norecopa has invested considerable resources in building a website of global 3R resources, coupled to an intelligent search engine. All these are available at one site: https://norecopa.no/. The search engine returns hits from all Norecopa's resources simultaneously. Filters can be applied to increase the relevance of the results. All searches and filters generate unique URLs, making it easy to document the searches which have been performed.

Reference

https://norecopa.no

VI-2-75

The 3Rs in action: How does Zoetis, a global animal health industry leader, promote animal well-being and the use of alternatives?

Sherry Vaughn[1], Catrina Stirling[2] and Torrie Condet[1]

[1]Animal Welfare Compliance, Zoetis, Kalamazoo, MI, United States; [2]Global Regulatory Affairs, Zoetis, Zaventem, Belgium
Sherry.E.Vaughn@Zoetis.com

Zoetis is ethically and legally obliged to rigorously evaluate new break-thru medicines and therapies.

The daily work of the Zoetis scientist involves exploration of *in vitro* alternatives. Discussions during protocol design focus on methods to refine *in vivo* work. Ethical review occurs prior to study start, to ensure humane care and use of animals for both internal and external *in vivo* work. Regional ethical review boards are responsible for evaluating alternatives to animal use and maximizing animal well-being during study activity. To recognize project teams and promote understanding that the appropriate use of alternatives is integral to ethical animal use and good science, Zoetis has put in place a global 3Rs Award program.

Zoetis scientists work directly with government regulators to increase the acceptance of *in vitro* models. Zoetis is providing veterinary vaccine leadership in the VAC2VAC project. The ultimate goal of the VAC2VAC project is to develop tests and approaches that will allow acceptance of the "Consistency Approach" for established vaccines. By providing veterinary vaccine leadership Zoetis promotes the use of alternatives externally.

VI-2-286

Challenges in developing and implementing 3Rs alternative methods in Argentina

Susana Gorzalczany

Pharmacology Department, Universidad de Buenos Aires, School of Pharmacy and Biochemistry, Ciudad Autónoma de Buenos Aires, Argentina
sgorza16@gmail.com

Argentinian research on alternative methods had always been carried out by isolated groups. In the last few years an increase in commitment to the 3Rs has been observed. Different groups from academia, biomedical researchers, professional societies, and industries are planning strategies to improve animal welfare and incorporate alternative methods for regulatory purpose but also for use in the life sciences. Institutional Animal Care and Use Committees have been incorporated in different public or private institutions to regulate use of animals in research, teaching and testing. Courses and symposiums are being offered to spread the principles of 3Rs in academic, industrial and regulatory fields, in order to move forward the implementation of alternative methods. Although funding program for replacing animal methods remain very limited, non-regulatory alternative used by academia try to force changes. Therefore, participation of relevant stakeholder is essential to reach the goal.

Session VI-3: Training for Humane Use of Animals in Veterinary Education and Biomedical Research

Co-Chairs
Jan van der Valk, 3Rs-Centre Utrecht Life Sciences, Utrecht, The Netherlands
Nicole Duffee, American Association for Laboratory Animal Science, Memphis, TN, United States

VI-3-421

Contribution of education and training to Culture of Care

Jan van der Valk

Dept. Animals in Science and Society, Fac. Vet. Medicine, Utrecht University, 3Rs-Centre Utrecht Life Sciences, Utrecht, The Netherlands
janvdrvalk@gmail.com

Directive 2010/63/EU on the protection of animals used for scientific purposes requires that EU Member States ensure that laboratory animal science (LAS) staff is adequately educated, trained and competent. "A working document on the development of a common education and training framework to fulfill the requirements under the Directive" was produced to guide the establishment of good quality courses and would, in the long run, promote the free movement of personnel. This process is facilitated by the Education and Training Platform for Laboratory Animals Science (ETPLAS) with stakeholders, members states, course providers and accreditation bodies, being represented. Focus of all training in LAS should be on the 3Rs. Although not specifically mentioned in the directive, crucial for the humane use of experimental animals is the correct attitude towards these ("Culture of Care"). It is essential that courses also contribute to this attitude.

References

https://www.etplas.eu/ (Education and Training Platform for Laboratory Animal Science)
http://bit.ly/2p7YYa9 (website of the LAS course at the Utrecht University)

VI-3-820

Professional development: Foundation of a strong animal care and use program

Nicole Duffee[1] and Ann Turner[2]

[1]Education Scientific Affairs, AALAS, Memphis, TN, United States; [2]AALAS, Memphis, TN, United States
nicole.duffee@aalas.org

The animal research field has gotten more sophisticated with the use of genetically engineered animals, biohazardous agents, and advanced technologies; moreover, those who work with the animals in the laboratory setting have one of the most consequential assignments in the research arena. Trained and competent personnel are the foundation of any animal care and use program. Individual career success is a combination of education, experience, continuing competence, professional development and personal commitment. Integration of training and certification into programs demonstrates institutional commitment to quality research. It also enhances the program by providing staff with knowledge and training to address problems and situations that arise and to perform their job in a professional and effective manner. Professional development programs offered through the American Association for Laboratory Animal Science, including the AALAS Learning Library and the AALAS certification process, will be presented.

VI-3-788

Requirements and recommendations on education & training around the world – Competence and performance standards

Javier Guillen

AAALAC International, Pamplona, Spain
jguillen@aaalac.org

Requirements on education and training exist in a number of countries or regions. However, many of them are generic; only a few consider learning outcomes associated to specific functions and include supervision of competence. Recommendations are produced by professional organizations to help ensuring that personnel at all levels are not only trained according to legal requirements, but are also competent in practice. In this context, institutions and competent authorities struggle to establish mechanisms to provide training and ensure and demonstrate competence at all levels of staff. Implementation of performance standards, based on the outcome rather than on the method used to achieve it, should be the way but, how can these be applied in practical terms? A clear definition of the expected outcome and a method to assess if this has been achieved, are needed.

VI-3-248

ReThink3R – Design Thinking Workshops for Young Scientists

Annemarie Lang[1,2,3], Laura Behm[2,4] and Cecilie Hilmer[5]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Berlin-Brandenburg School of Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany; [3]German Rheumatism Research Centre, Berlin, Germany; [4]Branch Bioanalytics and Bioprocesses, Fraunhofer Institute for Cell Therapy and Immunology IZI, Potsdam, Germany; [5]Unit for Bioentrepreneurship, Department of Learning, Informatics, Management and Ethics, Karolinska Institutet, Stockholm, Sweden

annemarie.lang@charite.de

The combination of numerous obligations, time constraints and inflexible research structures often leaves little room for young scientists performing animal experiments, to address the 3R topic and ethical concerns in a satisfying manner. In order to overcome this predicament, we are driven to offer interested scientists the possibility of dealing with the implementation of the 3Rs in various contexts through interactive Design Thinking Workshops. Design Thinking is an innovation method that combines both analytical and creative methods to find user-centered solutions in an iterative process. Here, we will present the results of the first five workshops that have been performed so far at different graduate schools and institutions in Berlin. This new workshop approach guides scientists through a difficult and emotionally complex topic and aims at training scientists in teamwork, an open-mindset and creative confidence – facilitating a change within the scientific community including awareness for the 3Rs.

VI-3-55

Collaborating in laboratory animal science education and training: LAS interactive, an integrative online platform

Nicole Linklater, Kirsten Grimpo and Cornelia Exner

Philipps Universität, Marburg, Germany

linklater.n@uni-marburg.de

The webpage "LAS interactive" is a third-party funded collaborative approach in laboratory animal science education, that enables experts from the different areas relevant to LAS to share their collective expertise within a common platform. Contributors of LAS interactive are individuals from e.g. Max Planck Society, German Primate Center, Cost Action Primtrain, LaNIV, universities and the industry. The platform contains information on different animal species and scientific techniques but addresses the ethics underlying the use of animals in research, animal welfare and the promotion of the 3Rs as well. The legal context is based on EU legislation but incorporates differing national requirements where appropriate. Procedures are illustrated by pictures and videos of live animals or by using teaching alternatives and interactive elements.

Future forums will enable the exchange of ideas and a resources page will be available where people can share teaching material. In the next step, we will expand the platform to also include descriptions and protocols of alternative methods, thus truly integrating the LAS and alternative methods communities.

Reference

http://las-interactive.de

VI-3-719

Lab animal welfare, science and medicine in African developing countries: Past five years

Francis Fakoya

St. George's International School of Medicine, Northumbria University, Newcastle upon Tyne, United Kingdom

ffakoya@sgu.edu

An earlier report indicated that many African investigators, including students and some research administrators are aware and agree that it is of paramount importance to treat lab animals humanely for the sake of the science they hope to present after all the efforts. Recently, many more are willing to participate in training programs to acquire further knowledge, skills, but in particular, a reorientation towards their approach to lab animals. Animal Care and use in Research, Education and Testing, (ACURET.ORG), incorporated in Nigeria, is promoting humane animal care and use for scientific purposes in developing countries, with a year 2020 target to reach every African Country. Through two international workshop/conferences, and an Independent Training program, supported by a few local and international organizations, ACURET has reached 245 attendees from four African countries. With the proposed hybrid online higher Education program, accreditation for four regional facilities across the continent, and an African Regional Consultative Network on Lab animal welfare, science and medicine, the number of Africans skilled in humane animal care and use will be raised to an appreciable level sufficient to participate in the global harmonization discuss in lab animal welfare.

Session VI-4: Innovative Teaching and Training Methods Using Alternatives

Co-Chairs
David Dewhurst, University of Edinburgh, Edinburgh, United Kingdom
Nick Jukes, InterNICHE, Leicester, United Kingdom

VI-4-364

The Virtual Pharmacology Lab – An online repository of free educational alternatives for practical pharmacology teaching

David Dewhurst and Ross Ward
Medicine, University of Edinburgh, Edinburgh, United Kingdom
d.dewhurst@ed.ac.uk

An online repository of free-to-use (educational Creative Commons license) "alternatives" learning objects (LOs) has been developed to assist university faculty in teaching pharmacology practical classes that frequently use live animal preparations. The 650+ metadata-tagged LOs were acquired by disaggregating existing multimedia simulations developed by the authors (http://www.sheffbp.co.uk) and include: data traces from experiments; (HTML) text descriptions; images, diagrams; video; interactive student tasks; self-assessments. Users browse or use a keyword search facility to find individual LOs each of which has associated descriptive text, a web link (url), the code to embed them into webpages/online content, and a preview (e.g. image, animation). The granularity of the LOs enables faculty to tailor the content of their teaching materials more readily. Summary website usage statistics will be presented together with examples of teacher-created e-books illustrating how the LOs may be used.

VI-4-189

ExPharm Pro – A computer assisted learning software for undergraduate students

Ramasamy Raveendran
Department of Pharmacology, JIPMER, Pondicherry, India
drravee@gmail.com

ExPharm Pro is an online software package for simulating animal experiments in pharmacology. It consists of five experiments namely the effect of drugs on frog heart, dog blood pressure-heart rate, frog esophagus and rabbit eye and bioassay of histamine using guinea pig ileum. These experiments are available in two modes namely tutorial and examination modes. The tutorial mode includes detailed instructions. The animal tissue/whole animal along with the equipment set-up is displayed on the screen for testing the drug effects. On application of drugs, the responses appear on the screen in realistic animated sequences. The data obtained by the student can be recorded in a table and a few questions will be displayed for the students to answer. The data and the answers are stored on the server and managed by an in-built students' management system. The examination mode displays tasks to be carried out by the student by choosing and doing an appropriate experiment. The answers and the steps carried out will be stored on the server for the teacher to evaluate the same. This software which is widely used in India has many more features and will be demonstrated to the delegates.

VI-4-83

CAAT Academy: Hands-on training in 3Rs: A tentative to fill in the gap

Francois Busquet
CAAT-Europe, Konstanz, Germany
caat-eu-policy@uni-konstanz.de

Over the last thirty years, dozens of validated alternative test methods exist in the EU and even more thanks to ICATM collaboration. Nevertheless, when one looks at the number of testing proposals submitted to REACH it is clear these methods are not being put to sufficient use. While ad-hoc events, tailor-made training, webinars, and scientific meetings regularly provide training in these new methods, more efforts should be invested into "after-sales" services to disseminate the emerging technologies and reach new audiences. The European Commission and the member states are actively filling the gaps in training via EU research programs such as Horizon2020, and the innovative medicines initiatives. This presentation will illustrate the mission of CAAT Academy's 3Rs training to increase the use of validated alternative methods and provide feedback and lessons learned since its creation in 2016 on the last six hands-on trainings which gathered in total approx. 80 participants in the lab and via webinars. Last, the sustainability of such initiatives will be described and the objectives of the medium-term announced.

VI-4-510

Use of the Elnady Technique for preserving specimens in education and training

Fawzy Elnady
Cairo University, Cairo, Egypt
elnady@cu.edu.eg

Seeking to avoid the harmful use of animals is a necessity for veterinary education and training. Alternatives such as computer software and virtual reality, manikins and models, and plastinated specimens, are powerful training tools but may not always provide sufficient hands-on experience. The Elnady Technique, a modified form of plastination, is simple and inexpensive. The developed specimens are realistic, dry, durable and flexible. The potential of using such specimens is broad. Organs, systems and whole cadavers can be developed for basic anatomy and dissection. For clinical skills, the technique has been successfully used in surgical suturing, upper respiratory endoscopy in the horse, dystocia in the cows and mare, amongst other procedures. It can also support the study of embryology, pathology, parasitology and internal medicine. Using a body donation program, animals that die naturally or in accidents, or that are euthanized for medical reasons, can be preserved with the Elnady Technique. This can help ensure a sufficient number of specimens for veterinary education and training, and contribute to the ending of the harmful use of animals.

VI-4-143

Evaluation of educator and student use of and attitudes toward dissection and dissection alternatives

Peggy Osenkowski, Marcia Kramer and Peggy Cunniff
NAVS, Chicago, IL, United States
posenkowski@navs.org

Animal dissection has been routinely practiced in American biology classrooms for decades. With technological advancements, more states adopting student choice measures, and increased awareness about ethical concerns surrounding dissection, many useful dissection alternatives have been developed. To understand the current use of animal dissection and alternatives, and attitudes toward the practices, a nationwide survey of middle and high school biology teachers and students was conducted. A similar survey was administered to students and mentors of the 2016 Intel International Science and Engineering Fair, the world's largest international pre-college science competition. Survey results, as well as suggested strategies to reduce animal use in education, in line with the 3 R's principle, will be shared.

Reference

Osenkowski, P., Green, C., Tjaden, A. and Cunniff, P. (2015). Evaluation of educator and student use of and attitudes toward dissection and dissection alternatives. *The American Biology Teacher 77*, 340-346.

VI-4-134

Understanding the cultural factors that contribute to the lack of uptake of non-animal alternatives for dissection in secondary education

Elisabeth Ormandy
Animals in Science Policy Institute, Vancouver, BC, Canada
elisabeth@animalsinscience.org

Advocacy groups have worked on the issue of animal dissection for many years, however, the pace of change is slow and animals are still widely used for dissection in North American secondary science education. This practice still occurs despite evidence that non-animal alternatives: 1) are often superior in educational merit compared to dissection; 2) are more economical; and 3) provide a safe, inclusive educational experience; and despite the ethics-based, Three Rs argument that if suitable non-animal alternatives exist then they must be used. We sought to better understand why these practical and ethical arguments have not led to greater uptake of alternatives. We surveyed science teachers in British Columbia, Canada about their views on dissection to identify possible points of resistance to non-animal alternatives. This presentation will share preliminary results and offer a discussion of possible steps that can be taken to shift the culture of science teaching so that non-animal alternatives are more readily adopted.

Session VI-5: Acceptance and Implementation of the 3Rs in Asia

Co-Chairs
Tsutomu Kurosawa, Kagoshima University, Sapporo, Japan
Jae-Hak Park, Seoul National University, Seoul, South Korea

VI-5-795

3Rs implementation to Japanese regulations

Tsutomu Kurosawa
Kagoshima University, Chuo-ku, Sapporo, Japan
japanawr@gmail.com

The main regulation for animal welfare is Animal Welfare Act (The Act) in Japan. The Act was revised several times since it was amended in 1973. The revision of The Act in 2005, 3Rs were stated. Refinement is the mandatory issue but Reduction and Replacement may be just statements without penalties for these two Rs violation. Japanese regulatory system is relied on the notifications by the ministries. The ministry of health notified JaCVAM and ICATM as important references in alternative test methods for cosmetics. JaCVAM published many validated alternative methods in its website such as corrosion test, sensitization test, skin irritation test, eye irritation test and pyrogen test etc. The most of industries now refer this website for their developing activities. The most recent notifications include alternative test methods for medical devices, pharmaceuticals and regeneration medicinals after the revision of the Pharmaceutical Affairs Act in 2014.

VI-5-93

Assessing current practice of the Three Rs principles: A national survey in Korea

Byung-in Choe[1], Gwi-hyang Lee[1,2], Yuri Hwang[1], Moon Seok Yoon[3] and Lynette Hart[4]
[1]Nicholas Cardinal Cheong Graduate School for Life, The Catholic University of Korea, Seoul, South Korea; [2]BIC Study Foundation, Seoul, South Korea; [3]Animal Protection & Welfare Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries, Gimcheon-si, South Korea; [4]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, United States
bichoe@catholic.ac.kr

Scientists planning research involving the use of animals are required to examine the possibilities for replacement, reduction or refinement (Three Rs) by Korean law. Through this process, researchers could prevent unintended duplication of animal experimentation and may acquire improved methods: to reduce or replace animal use and refine procedures to minimise pain and distress to animals. Based on our national survey of Korean IACUC members and researchers in 2012, assessing the practice of literature searching to comply with the need to use Three Rs alternatives, 12% of respondents had no relevant experience on the literature search on Three Rs alternatives. 25% of respondents were not aware of how or where to find information on alternatives to animal use. The survey showed that 72% of respondents used only PubMed (Medline) and Google search engines to look for Three Rs alternatives to avoid unnecessary duplication.

There is little specific information or resources on Three Rs alternatives readily available in the Korean language. The term, "*alternatives*", is often misinterpreted as only "*replacement*" in Korean. Searching for Three Rs alternatives is not a structural part of the research process, and searching skills are limited.

To help with this language barrier and acquiring basic information on the Three Rs, the authors developed an independent, nonprofit platform website and published three Korean guide books on the Three Rs concept and searching guide. They are a Korean version of "*The Three Rs and Humanity Criterion*", written by Professor Michael Balls in 2009 and published by FRAME, *a 2nd Korean translation version of "The 3Rs Good Practice: Effective Search Strategies to comply with the 3Rs"*, published by the Joint Research Centre's European Union Reference Laboratory for Alternative to Animal Testing (EURL ECVAM), and Korean Supplement referred on Korean research culture.

This paper summarizes key findings from our national survey conducted among Institutional Animal Care and Use Committees (IACUCs) in May 2016. A link to the online questionnaire was set up and distributed to IACUCs in Korea. There were 347 IACUCs registered to the Korean government agency in 2015. The Korean government agency (Animal Protection & Welfare Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries) sent out a supporting letter to each IACUC asking them to encourage participation in the survey within their institutions. In addition, complementary Korean guide books, published by the authors, were delivered to each respondent who completed the questionnaire. The questionnaire comprised four categories: 1) general information, 2) knowledge of Three Rs principles and applying Three Rs in searching practice, 3) searching skills and education status, 4) interesting methods for further training. Some questionnaires were comparable to the earlier survey results conducted by Choe et al. (2012). To gain more insight into how Korean IACUCs, researchers, educators, and administrative staffs were aware and applying Three Rs information in their work, four different types of questionnaire were prepared based on the participants' roles.

A total of 510 respondents filled out the questionnaire, representing 84 IACUCs, 296 researchers, 28 educators, and 102 administrative staffs. Their affiliated institutions comprised: government and public (29.6%), academia (36.3%), medicine (5.9%), industry (23.1%), and 5.1% from other institutions. The results were analysed by the professional survey consultant company, Insight Korea Research & Consulting. The findings of this survey include that fewer than half of the researchers (47.6%) were familiar with methods for searching the literature for information on Three Rs and they find this to be a challenging task. Most respondents (82.7%) recommended that specific training on Three Rs searching skills should be included in the required training course. Therefore, it is important to consider whether Three Rs information is readily available and whether scientists are effectively accessing it. The ability to access and implement information on the Three Rs is essential for ethical and scientific reasons, because this can improve animal welfare and scientific outcomes, as well as to the saving of resources. It can prevent the unnecessary duplication of studies, improve experimental design, and ensure that existing and new alternative methods are used as widely as possible. Promotion and protection of laboratory animals comprise one of the core competencies of well-educated personnel involved in their use.

VI-5-801

The 3Rs in Singapore: Developing supportive infrastructure and networks for world-class animal-based research and teaching

Jassia Pang
National University of Singapore, Singapore
jpang@nus.edu.sg

In the early 2000s, the National Advisory Committee on Laboratory Animal Research was established in Singapore to develop guidelines on the care and use of animals for scientific purposes. These guidelines were released a year later and have become the primary standard for local regulatory agencies and institutions. In adopting this document, institutional animal care and use committees were formed which have helped increase awareness in considering the 3Rs whenever the use of animals is necessary. Since then, we have played on the advantages of being a small country, making progress in streamlining animal-based research and teaching in Singapore. From the establishment of national breeding centres and imaging facilities to standardising health statuses between organisations, this has not only eased collaborations but also reduced the need for duplication. Strong partnerships between the bigger animal facilities have also led to a greater pool of resources especially for training and lending expertise. These are some of the initiatives that exemplify the commitment of the country as a whole to the responsible use of animals for scientific advancement.

VI-5-45

Comparison of application between EpiSkin™ and Epikutis® skin model in skin irritation and skin corrosion assay

Jingsong Zhang, Jing Sang, Licheng He, Hua Kang, Rong Kuang and Yang Li
Zhejiang Institute for Food and Drug Control, People's Republic of China
zjsonly@hotmail.com

Currently there were two types of reconstructed human skin models (EpiSkin™ and EpiKutis®) in Chinese Market. Both models were made of reconstructed human epidermis (obtained from human derived non-transformed epidermal keratinocytes) which closely mimics the histological, morphological, biochemical and physiological properties of the upper parts of the human skin. Here we compared two models in skin irritation and skin corrosion assay *in vitro*. According to OECD Guide line 439 and 431, we compared two models in quality control, test method, results evaluation, transportation, etc. It is showed that there were differences in quality control (viability and barrier function), test method (dose and exposure time), results evaluation, operability and cost. Each model has its merits and demerits, but both models could meet the OECD Guide line requirement of sensitivity, specificity and accuracy in these two assays. It will be a good alternative to animal test in cosmetics toxicology test in China.

VI-5-562

The 3Rs change after 10 years KSAAE activities

Eui-Bae Jeung
College of Veterinary Medicine, Chungbuk National University, Seowon/Cheongju, South Korea
ebjeung@chungbuk.ac.kr

In 1999, the concept of "3R" was officially introduced from 3rd World Congress on Alternatives to Animal Use in the Life Sciences, and Korea also established the 2007 Korean Society for Alternative to Animal Experiments (KSAAE) in order to play a pioneering role in research, development, education and supervision to suggest the principle of proper animal use. Recently, a public interest in protecting animals through legislation for protection and welfare of animals has increased greatly in Korea, and in particular in the fields of assessment of safety and toxicity, new options and alternatives for restriction in animal use and protection and welfare of animals are urgently required. Although the use of laboratory animals in safety and toxicity studies is somewhat inevitable, the development of humanitarian alternative test methods based on the respect of life is consistently necessary. Along with the development of advanced technology, we hope that in the future we will be able to predict and evaluate safety and toxicity without using laboratory animals, so we want to constantly make an effort.

Since the animal use in testing European cosmetic raw materials and products was banned in 2013, ban on animal testing in the field of cosmetics has been expanding worldwide, and Korea has also been unable to distribute or sell cosmetics made through animal experiments from February 2017. In addition, various activities are carried out to promote research on the alternatives to animal use, mainly by the National Institute of Food and Drug Safety Evaluation, and research on animal-free alternatives will be greatly expanded from now on.

In response to the needs of the times, KSAAE provides infrastructure for the research and development of alternatives to animals and a place where members from various fields can gather together and make assertive academic activities. We will continue to pay the necessary efforts to improve domestic research level to the world level.

Session VI-6: The 3Rs in Research and Funding Opportunities for 3Rs Research

Co-Chairs
Mardas Daneshian, CAAT-Europe, University of Konstanz, Konstanz, Germany
Kristina Adams, USDA, NAL, Animal Welfare Information Center, Beltsville, MD, United States

VI-6-779

Awareness of the economic potential of non-animal approaches mirrored by governmental funding schemes

Mardas Daneshian
CAAT-Europe, University of Konstanz, Konstanz, Germany
mardas.daneshian@uni-konstanz.de

The scientific progress of non-animal methodologies in the last decade reveals how the near future of safety sciences and basic research may look like. The evolution of these methodologies implies the combination of *in vitro* and *in silico* approaches and the integration of information from manifold of sources in order to predict safety issues and to tackle questions from basic research. Contrary to animal-based approaches, non-animal methodologies approaches comprise manifold of aspects which can be patented and licensed. Thus, there is a huge economic potential of non-animal methodologies. The presentation will focus on governmental funding schemes and measures to correlate these schemes with the awareness of the economic potential of non-animal approaches.

VI-6-792

Finding funding opportunities in 3Rs research

Kristina Adams
USDA, NAL, Animal Welfare Information Center, Beltsville, MD, United States
kristina.adams@ars.usda.gov

For many of the same reasons that alternatives information in the literature is often difficult to tease out, finding funding opportunities for 3Rs focused research can be difficult. In the field of animal research, and especially when looking for 3Rs information, searching requires sophistication on the part of the searcher that for the most part does not exist. The US Department of Agriculture's Animal Welfare Information Center (AWIC) has long provided training on how to develop and implement effective search strategies for finding information in the literature about the 3Rs relevant to specific animal study proposals. In this brief talk, I will highlight some funding resources that are available for 3Rs research. I will also discuss strategies for finding US federally funded research projects that incorporate the 3Rs, even if it is not the primary focus of the grant.

VI-6-600

European Commission support for research into the 3Rs

Christian Desaintes, Bernard Mulligan and Arnd Hoeveler
European Commission DG Research & Innovation, Brussels, Belgium
christian.desaintes@ec.europa.eu

The European Commission (EC) has supported long-term 3Rs research through successive EC Framework Programmes for Research and Innovation, including the current seven-year programme *Horizon 2020 (H2020*: 2014 to 2020). During the last decade, EC funding has averaged more than EUR 35 million per year to new research projects. During 2012-2017, seventy research projects were running at various stages of implementation, with EUR 350 million from EC programmes. As part of this effort, fourteen projects were co-financed by industry (by the Innovative Medicines Initiative or Cosmetics Europe), providing an additional EUR 120 million. The main research activities are targeted at better and more cost-effective safety and efficacy testing of chemicals, nano-particles, vaccines and drugs. In this context, the EU-ToxRisk project, funded with EUR 30 million under *H2020*, has the ambition to open a new era of safety sciences. Further opportunities for funding in *H2020* will be presented.

VI-6-806

Funding research and validation of alternative methods in the United States

Warren Casey
NTP, Research Triangle Park, NC, United States
warren.casey@nih.gov

Funding opportunities for the validation of alternative methods in the US. Small businesses involved in alternative methods development to apply for funding offered by the National Institute of Environmental Health Sciences (NIEHS). These awards are intended to support the validation of promising alternative test methods that replace or reduce animal use in toxicity testing/screening. Priority areas for alternative test method development and validation include (but are not limited to) ocular toxicity testing, reproductive and developmental toxicity testing, carcinogenicity testing, and acute toxicity testing. This talk will provide an overview of current funding opportunities for small businesses and other grant programs aimed at research which supports the 3Rs.

Session VI-7 (Part 1): The Importance of Experimental Design in Animal Experiments

Co-Chairs
Hanno Wuerbel, University of Bern, Bern, Switzerland
Nathalie Percie du Sert, NC3Rs, London, United Kingdom

VI-7-621

Risk of bias in animal research

Emily Sena
University of Edinburgh, Edinburgh, United Kingdom
sena.emily@gmail.com

Experiments conducted without taking measures to reduce the risk of bias do not fully realise their potential. Potential risks of bias in an experiment include threats to internal, external and construct validity and publication bias poses further limitations to the utility of animal research.

Systematic review of a non-random sample of *in vivo* studies suggests that only a third of studies report randomisation and blinded assessment of outcome and less than 1% report a sample size calculation. Studies not reporting measures to reduce the risk of bias were associated with reporting inflated effect sizes. In an assessment of publication bias in the *in vivo* stroke literature we estimate that 1 in 6 experiments remain unpublished and this leads overestimation of around 30% of reported treatment effects.

For animal research to be useful experiments need to be designed, conducted, analysed and reported with care taken to reduce the introduction of potential sources of bias.

VI-7-362

NC3Rs resources to improve the design and reporting of animal research

Viki Hurst and Nathalie Percie du Sert
NC3Rs, London, United Kingdom
viki.hurst@nc3rs.org.uk

The reproducibility of biomedical research using animals has come under scrutiny in recent years, and quality standards in the design, analysis and reporting of *in vivo* research have been flagged as concerns. The NC3Rs has been working in this area over the last ten years and led the development of two key resources to support researchers and improve the design, analysis and reporting of *in vivo* experiments. The ARRIVE guidelines consist in a 20 item checklist, which summarise the minimum information necessary to describe a study in a comprehensive and transparent manner. The guidelines cover the main aspects of a scientific publication and make recommendations on the reporting of the study design, experimental procedures, animal characteristics, housing and husbandry, and statistical analysis. Several studies are investigating the impact of the guidelines and their usability. The Experimental Design Assistant is a web application with a supporting website, which helps researchers design animal experiments, by increasing the transparency of the experimental plan, and providing feedback and dedicated support for randomisation, blinding and sample size calculation. The objective of these resources is to maximise the output of research using animals. Wide dissemination and uptake are essential to ensure the science emerging from animal research is fully exploited.

References

Cressey, D. (2016). Surge in support for animal-research guidelines. *Nature*. http://www.nature.com/news/surgein-support-for-animal-research-guidelines-1.19274

Cressey, D. (2016). Web tool aims to reduce flaws in animal studies. *Nature 531*, 128. http://www.nature.com/news/web-tool-aims-to-reduce-flaws-in-animal-studies-1.19459

Kilkenny, C., Browne, W. J., Cuthill, I. C. et al. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biol 8*, e1000412, doi:10.1371/journal.pbio.1000412 (2010)

VI-7-680

Make each subject count

Mandy Bergquist
GSK, Collegeville, PA, United States
mlbergquist@yahoo.com

Recent publications have highlighted poor quality, lack of reproducibility, and deficient reporting in preclinical research, concluding that the lack of rigor around the "generation and analysis of preclinical data is reminiscent of the situation in clinical research about 50 years ago." (Begley and Ellis, 2012). Inadequate robustness in design, analysis, and reporting contributes to high failure rates in translating preclinical results to clinical settings. Studies that adopt better statistical practices yield greater insight and more robust conclusions. Reviewing the current state of statistical practice in preclinical pharmacology reveals areas for improvement. A series of examples is then used to illustrate good statistical practices that can be widely used to improve the translation of results, to minimize the number of samples, and to ensure that researchers maximize the value of the information gained from each subject.

Reference

Begley, C. G. and Ellis, L. M. (2012). Raise standards for preclinical cancer research. *Nature 483*, 531-533.

Session VI-7 (Part 2): The Importance of Experimental Design in Animal Experiments

Co-Chairs
Hanno Wuerbel, University of Bern, Bern, Switzerland
Nathalie Percie du Sert, NC3Rs, London, United Kingdom

VI-7-629

The problems with small sample sizes

Katherine Button

Department of Psychology, University of Bath, United Kingdom
k.s.button@bath.ac.uk

The routine use of small sample sizes undermines the reliability and reproducibility of research findings. Small studies have lower statistical power and are therefore more likely to miss genuine effects, and any significant results have a higher likelihood of being false-positive or inflated estimates. The results from small studies are more variable and thus more susceptible the effects of questionable research practices such as p-hacking, hypothesizing after the results are known, and selectively publishing positive results. Such unreliable research is inefficient, wasteful and unethical. Adopting best-practices to improve reproducibility is therefore a priority. This requires attention to sound methodological design, using realistic effect size estimates to perform power calculations to determine the sample sizes needed to produce reliable, reproducible, and clinically meaningful results.

VI-7-363

Demonstration of the Experimental Design Assistant

Nathalie Percie du Sert and Viki Hurst

NC3Rs, London, United Kingdom
nathalie.perciedusert@nc3rs.org.uk

The Experimental Design Assistant (EDA; https://eda.nc3rs.org.uk) is a free web-based tool which was developed by the NC3Rs. It guides researchers through the design and analysis of *in vivo* experiments. The EDA allows you to build a stepwise visual representation of your experiment, providing feedback and dedicated support for randomisation, blinding and sample size.
Features include:
– Computer-aided design tool to develop a diagram representing the experimental plan
– Critical feedback on the experimental plan – using computer-based logical reasoning
– Statistical analysis suggestions
– Sample size calculation
– Randomisation sequence generation
– Support for allocation concealment and blinding
– Web-based resources to improve knowledge of experimental design and analysis

This demonstration will provide an introduction to the tool and provide guidance on getting started. There is no requirement for any previous knowledge of the EDA. Ultimately, the use of a tool such as the EDA will lead to carefully designed experiments that yield robust and reproducible data using the minimum number of animals consistent with scientific objectives.

Reference

Cressey, D. (2016). Web tool aims to reduce flaws in animal studies. *Nature 531*, 128. http://www.nature.com/news/web-tool-aims-to-reduce-flaws-in-animal-studies-1.19459

VI-7-597

Power failure: Larger sample sizes fail to solve the reproducibility problem in animal research

Hanno Wuerbel

Division of Animal Welfare, University of Bern, Bern, Switzerland
hanno.wuerbel@vetsuisse.unibe.ch

Reproducibility in animal research is alarmingly low. Various threats to reproducibility have been identified, including poor scientific rigor, low statistical power, analytical flexibility, and publication bias. However, reproducibility is mainly a function of external validity. Both theoretical and empirical evidence indicate that standardization generates spurious results accounting for poor reproducibility. Multi-lab studies with as few as 2 to 4 labs greatly increase the reproducibility of results, without a need for larger sample sizes. Therefore, heterogenization rather than standardization is needed to improve reproducibility. Importantly, larger sample sizes will not solve the problem. Quite to the contrary, higher statistical power yields results that are more precise but less accurate. More representative study samples to enhance external validity may thus be the only way out of the reproducibility crisis, helping to avoid wasting animals for inconclusive research.

VI-7-356

Effectiveness of education for better animal experiments and Reduction

Derek Fry

University of Manchester, Manchester, United Kingdom
dj@fry39.fsnet.co.uk

Concerns over poor study quality and experimental design have been well aired. Poor design is contrary to the principle of Reduction as it leads to animal use being higher or less effective than it could be. Raising awareness of the problem has been an important stimulus to improving matters but there also seems to be a need for better education in design. Workshops in experimental design that concentrate on key understandings and develop skill in identifying design faults and appropriate designs for different experimental questions have been run to address the problem, both as FRAME Training Schools and as many spin-offs, in various countries. To judge from the marked improvement between pre- and post-test performances these are effective, and there are also specific instances of alteration of study proposals or laboratory practice. This presentation will review this data and illustrate the ideas and educational approaches used in the workshops.

Reference

Fry, D. (2014). Teaching experimental design. *ILAR J 55*, 457-471. doi:10.1093/ilar/ilu031

Session VI: 3Rs in Academia

Poster Presentations

VI-17

Laboratory animal science course in Switzerland: Participants points of view and implications for organisers

Marcel Gyger[1] *and Fabienne Crettaz von Roten*[2]

[1]Center of PhenoGenomics, EPFL, Lausanne, Switzerland; [2]SSP, UNIL, Lausanne, Switzerland

marcel.gyger@epfl.ch

Switzerland has implemented a mandatory training in laboratory animal science since 1999, mixing alternative and live animal approaches, however large assessment of its effects has never been undertaken so far. The results from the analysis of participants of the Swiss Federation of European Laboratory Animal Science Associations accredited category B (i.e. European Union Function A, person carrying out procedures) compulsory courses in laboratory animal science in 2010, 2012, 2014 and 2016, showed that the participants fully appreciated all elements of the course. The use of live animals during the course was endorsed and explained by six arguments that invoke cognitive, emotional and foresight-enabling factors. A wide majority considered that the 3Rs principles were adequately applied during the course. Responses to an open question offered some ideas for improvements. This overall positive picture, however, reveals differentiated answers when considering different subpopulations in our sample (for example, scientists with more hindsight, scientists trained in biology, participants from Asian countries).

References

EU Directive 2010/63: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

FELASA website: http://www.felasa.eu/accreditation-boards/accreditation-board-for-education-and-training1/

VI-26

New technology advances the 3Rs in a transgenic animal core facility

Xin Rairdan and Rhonda Wiler

Transgenic Technology, Genentech, South San Francisco, CA, United States

xinr@gene.com

Genetically modified rodent models are important tools for biomedical research. With recent advancements of technology, *in vitro* methods can be utilized to completely replace or significantly reduce animal use in the process of creating or maintaining these models. Our facility had implemented CRISPR technology in generating rodent models that shortened the timeline to create the models and reduced animal use by 86%. Using recycled mice and rat for donor and recipient reduced number of animals ordered up to 71%. Replacement of live pups with cultured embryos for quality control of cryopreservation decreased animal use by 88%. Using *in vitro* methods, such as, HTN-Cre Recombinase for allele conversion reduced animal use by a minimum of 76%. These well-established methods that apply the 3Rs principles should be the standard for any transgenic core facilities to reduce animal use.

VI-70

In vitro systems toxicology assessment of exposure to aerosol from a carbon heated tobacco product as compared with exposure to cigarette smoke: The impact on nasal and small airway epithelial cultures

Anita Iskandar, Yannick Martinez, Carole Mathis, Patrice Leroy, Florian Martin, Laura Ortega Torres, Shaoib Majeed, Keyur Trivedi, Stefan Frentzel, Emmanuel Guedj, Celine Merg, Ashraf Elamin, Nikolai Ivanov, Manuel Peitsch and Julia Hoeng

Philip Morris International, Neuchatel, Switzerland
Anita.iskandar@pmi.com

Toxicological assessment of tobacco products should provide relevant indication of the health risk for humans. Advances in tissue engineering have allowed the development of *in vitro* organotypic cultures with an air-liquid interface, thus permitting a direct exposure to inhaled chemicals. Using human organotypic nasal and small airway epithelial cultures, this study assessed the impact of an aerosol from a carbon heated tobacco product (CHTP) 1.2 – a potential modified-risk tobacco product – compared with cigarette smoke (CS) at similar nicotine levels. Various endpoints including cytotoxicity, histology, ciliary beating function, cytochrome P450 1A1/1B1 activity, and secreted pro-inflammatory mediators, were complemented by a systems biology analysis of the transcriptomes to assess the exposure impact at different post-exposure time points. The overall data demonstrate a substantially reduced biological impact of CHTP1.2 exposure compared with CS in both nasal and small airway cultures.

VI-81

Is Twitter appropriate to disseminate 3Rs work?

Francois Busquet and Ilija Prackovski

CAAT Academy, Ixelles, Belgium
caat-academy@uni-konstanz.de

Twitter social network was funded in 2006 and claims 319 million active users in 2016. Approximately 500 million tweets are tweeted each day. Thanks to the development of a specific twitter application, more than 430,000 tweets were collected from October 2014 to April 2017 based on the following hashtags: #animaltesting OR #animalfreetesting OR #animalfreetests OR #animalexperiments OR #3Rs OR #3R OR #BeCrueltyFree OR #endanimaltesting OR #stopanimaltesting OR #stopvivisection. Based on this unprecedented analysis, the authors were able to 1) identify the absolute number of users as well as its variation over the last three years, 2) the popular tweets and users 3) extracting new hashtags from pre-defined ones. Moreover, a twitter sentiment analysis in R-language was performed resulting in classification emotion content of the tweets ranging from anger to surprise or joy with a majority of fear. Nevertheless, the positive sentiment over time is almost always superior to the negative sentiment. Last but not least, the author's hypothesis whether 3Rs activity in twitter is reflecting the progress, the workload and the key events of the community is tested.

VI-100

Preclinical innovation and patient safety: A collaborative approach to supporting innovative science and replacing preclinical animal tests

Elizabeth Baker

Physicians Committee for Responsible Medicine, Washington, DC, United States
ebaker@pcrm.org

Preclinical testing is critical to safe and effective drug development. Traditional *in vitro* and animal tests fail over 95% of the time. Many modern, human-focused tests exist, and others, such as tissue chips, computer simulations and 3D printed organs, continue to emerge, but are not widely adopted due to scientific, legal and training issues. The Preclinical Innovation and Patient Safety initiative fosters collaboration across stakeholders, including federal agencies, patient, research and health organizations, academia, tech companies, and the pharma industry. We recently hosted a roundtable and follow up meeting to discuss opportunities to advance innovative, human-focused science, law and policy. Next steps include a publication with recommendations, projects to improve validation and training, and modernizing agency regulations. Working together to achieve common goals, PIPS helps ensure safer and more effective medicines are more quickly developed with less animal testing.

VI-101

Effect of essential oils contained linalool on skin sensitization using human Cell Line Activation Test

Yuji Yoshiyama, Maki Doi and Hideto Ariumi

Kitasato University, Tokyo, Japan
yoshiyamay@pharm.kitasato-u.ac.jp

The skin sensitization potential of chemicals has traditionally been evaluated *in vivo* according to OECD testing guidelines in guinea pigs or the mouse local lymph node assay. The human Cell Line Activation Test (h-CLAT), an *in vitro* skin sensitization test, is based on the augmentation of CD86 and CD54 expression in THP-1 cells following exposure to chemicals. Recently, the antinociceptive activities of linalool and linalool acetate are found as major components of essential oils in various aromatic plants, however, safety of essential oils on the skin sensitization is unknown. We investigated the skin sensitization potential of essential oil contained linalool according to OECD TG 422 E. An h-CLAT prediction is considered POSITIVE if at least one of the following conditions is met in 2 of 2 or in at least 2 of 3 independent runs, otherwise the h-CLAT prediction is considered NEGATIVE. The RFI of CD86 is equal to or greater than 150% at any tested concentration (with cell viability ≥ 50%), or the RFI of CD54 is equal to or greater than 200% at any tested concentration (with cell viability ≥ 50%) is considered POSITIVE. The essential oil of thyme linalool presented much higher values over 200% of CD54. We suggest that thyme linalool containing thymol and carvacrol may play an important role in skin sensitization.

VI-141

Providing freely available 3Rs information: Humane Endpoints website, Interspecies Database and FCS-free database

Saskia Kliphuis

Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
s.kliphuis@uu.nl

The 3Rs-Centre Utrecht Life Sciences (ULS) (www.uu.nl/3RsCentreULS) has initiated the 3Rs Database Programme, which aims to make 3Rs info freely available, thus contributing to the implementation of the 3Rs in research. The programme has adopted the Interspecies Database and the Humane Endpoints website.

The Interspecies Database (www.interspeciesinfo.com) provides insight into physiological, anatomical and biochemical parameters of different animal species and humans. With the database, researchers can design their experiments smarter with respect to the choice of an animal model. This could lead to a reduction in the number of experimental animals.

The Humane Endpoints website (www.humane-endpoints.info) provides information and training modules on how to recognize and apply humane endpoints in laboratory animals. This helps to prevent unnecessary pain and distress in the animals. Therefore, the website contributes to refinement.

In addition, a database on fetal calf serum (FCS)-free media will be launched in 2017. This database allows researchers to exchange information on the quality of growth media that do not contain FCS. This website will contribute to replacement.

VI-160

Modified DPRA for testing poorly water-soluble substances using immobilized peptide and a thiol or amino group indicators

Minamino Yuuki

Konan University, Kobe, Japan
tottu1227@yahoo.co.jp

A direct peptide reactivity assay (DPRA) is the most promising method as an effective alternative to animal testing for skin sensitization (OECD, 2015). DPRA assesses the binding of chemical substances to testing peptides by HPLC. DPRA hardly offers accurate measurements for poorly water-soluble substances, and HPLC takes time in comparison with other methods. This precludes the ready assessment of numerous types of samples in a short period of time. To solve these problems, we conjugated the DPRA peptides to amphiphilic resin and utilized thiol or amino group indicators for detecting amount of unreacted peptides (Alaoui et al., 2005). We examined poorly water-soluble substances by our modified DPRA. The results suggested that our modified DPRA was able to appropriately assess poorly water-soluble substances that conventional DPRA was unable to assess. Thus, this study successfully improved the conventional DPRA. Our method is simple and easy to handle and allows a high throughput for testing skin sensitization.

References

Alaoui, I. M., Menzel, E. R., Farag, M. et al. (2005). Mass spectra and time-resolved fluorescence spectroscopy of the reaction product of glycine with 1,2-indanedione in methanol. *Forensic Sci Int 152*, 215-219.

OECD (2015). Test Guideline 442C: In Chemico Skin Sensitisation, Direct Peptide Reactivity Assay (DPRA) (2015).

VI-163

(Q)SAR evaluation of the genotoxicity of impurities in drugs and the resulting control under ICH M7 guideline

Qingfen Zhu[1], Xia Wei[1] and Defu Hu[2]

[1]Department of Pharmacology & Toxicology, Shandong Institute for Food and Drug Control, Jinan, Shandong, People's Republic of China; [2]Shandong Institute for Food and Drug Control, Jinan, Shandong, People's Republic of China
zhuqingfen@hotmail.com

A key aspect of ICH M7 guideline is the proposed use of (quantitative) structure activity relationship [(Q)SAR] predictions as a substitute for an experimental Ames assay. According to ICH M7, we assessed the genotoxicity of several drug impurities in CHP 2015. Two (Q)SAR prediction methodologies were applied, of which, Derek is expert rule-based and Sarah is statistical-based. *In silico* evaluation showed that Hydroquinone was a class 1 GTI, 5-hydroxymethylfurfural was a class 2 GTI. The potential exposure was calculated based on the dosage. Then the limit of a genotoxic impurity was evaluated. The limit of them should be reduced to an acceptable level. 2-Methyl-5-nitroimidazole and Impurity I in nifedipine were classified as class 3, while the results of the Ames test were positive and negative, respectively. And they should be controlled as a genotoxic and non-genotoxic impurity, respectively. Impurity I in ranitidine hydrochloride was classified as class 4. Dextrodropropzine was classified as class 5. Both of them should be controlled as non-genotoxic impurities.

Reference

ICH (2014). M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk.

VI-168

Learning with virtual patients in the field of "Health management in pig farms" – "CASUS" a case-based e-learning model as a complement tool in the practical veterinary education and advanced training as well as in terms of the 3R's

Sebastian Spiegel[1], Florian Spiegel[1], Svenja Lösken[2], Andrea Düngelhoef[2], Elisabeth große Beilage[2], Christin Kleinsorgen[3], Jan Ehlers[4] and Michael Wendt[1]

[1]Clinic for Swine and Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [2]Field Station for Epidemiology, Bakum, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [3]E-Learning Department, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [4]Faculty of Health, University of Witten/Herdecke, Witten/Herdecke, Germany

spiegel.s@gmx.de

Using an interactive, case- and web-based tutorial "CASUS – Learning with virtual patients" veterinary students are enabled to test their individual learning success as well as to prepare themselves for practical procedures and exams.

Herd visits and checks on pig farms were simulated by the University of Veterinary Medicine Hannover, Foundation Germany in e-learning-based cases. Due to the increased hygienic standards in pig farms, it is no longer possible to provide each student with the possibility to visit such farms. To close this gap, already eight cases were designed and included in the curriculum.

Integrating the 3R's thereby takes on steadily increasing importance. Using the tutorial "CASUS", following practical procedures can be performed faster, safer and more experienced, leading to less stress in animals (refinement). Furthermore, the number of animals could be reduced (reduction), because fewer animals are needed for the practical exercises after better preparation.

VI-178

Use of clay modeling to teach human anatomy at the secondary school level: Student attitudes and outcomes

Emma Grigg and Lynette Hart

Vet Med – Population Health and Reproduction, University of California – Davis, Davis, CA, United States

ekgrigg@ucdavis.edu

This study is testing the effectiveness and appeal of clay models vs. dissection in teaching human anatomy to high school students. Phase 1 assessed student attitudes and learning outcomes when using clay models.

Students (n = 64) enrolled in an anatomy course (that formerly used cat dissection) completed an opinion survey on dissection, and an anatomy knowledge assessment, before and after the lab. No difference was found between attitudes towards dissection before vs. after the lab ($p = 0.46$). However, 92% of students described the models exercise as useful, and 84% as enjoyable. Before the lab, 27% indicated that they would choose to use clay models (63% chose dissection). After the lab, 41% chose models, 54% dissection. Student knowledge scores increased significantly following the lab ($p < 0.0001$).

The preliminary results are encouraging, in students expecting a dissection exercise but instead using models. Phase 2 will compare attitudes and outcomes using dissection vs. clay models.

VI-190

InVitro+Jobs – Information platform, working group network and job board for animal-free research

Christiane Hohensee, Christiane Baumgartl-Simons and Christina Ledermann

Federal Association of People for Animal Rights Germany (PARG), Berlin, Germany
hohensee@invitrojobs.com

In the last 20 years many new animal-free testing methods have been developed. However, only few methods have achieved the ultimate aim of actually replacing animal experiments. For the general public, an understanding of new suitable methods is becoming increasingly difficult and sometimes needs help finding suitable explanations.

The portal *InVitro+Jobs* is a project of the German Federal Association of People for Animal Rights to promote modern research without animal use. The bilingual platform *InVitro+Jobs* provides pertinent background reports on the path from research and development to acceptance and implementation in test guidelines. The goal is to promote discussion by illustrating how and why a particular method may not yet be adequate, what is lacking and what demands must be met to facilitate development and financial support. The *InVitro+Jobs* approach offers a critical but unbiased neutral scientific perspective. The readers are universities, scientists, young researchers, government agencies and the general public with an interest in scientific issues.

Young researchers and interested parties can get a quick overview of working groups dealing with animal-free research, as well as with vacancies and thesis work in this field. Contacts can be found quickly and easily. As an offer for journalists we can recommend an academic expert from our research group list. We regularly publish news on research results obtained without animal testing. Under the heading "Working group – a portrait" we present scientific teams and companies, and discuss current developments in greater detail. Current major topics are disease-on-a-chip models, organ-on-a-chip models, imaging techniques or induced pluripotent stem cells. In the future, relevant potential developments in this area could help to avoid the killing of many animals, particularly in pharmaceutical and biomedical research.

VI-210

Gaining confidence in alternative animal health monitoring programs: How do they compare to traditional soiled-bedding sentinel programs

Christie Birkle and Alan Vest

Vertex Pharmaceuticals, San Diego, CA, United States
christie_birkle@vrtx.com

In support of the 3Rs, our facility aimed to replace the traditional soiled-bedding sentinel program as a means of animal health surveillance. Although environmental PCR sampling has been used for years, it has not entirely been accepted as a replacement for traditional sentinel programs throughout the animal husbandry community. Our goal was to prove that environmental health monitoring known as PRIA (PCR Rodent Infectious Agent) was comparable to soiled-bedding sentinels. Due to a diverse health monitoring need, we quickly discovered that each colony would require a specialized program. By customizing each colony, we selected the appropriate alternative health monitoring program to optimize the detection of infectious agents. After a year of comparison studies, we were able to determine that alternative testing methods were more effective at detecting infectious agents than soiled-bedding sentinels; leading our facility to adopt an alternative animal health monitoring program.

VI-242

The Danish 3R-Center

Rasmus Nielsen

The Danish 3R-Center, Glostrup, Denmark
ranon@fvst.dk

Projects: An important part of the Danish 3R-Center's activities is to fund 3R-research projects http://en.3rcenter.dk/research/projects/

Symposium: Our annual international symposium is a significant event in efforts to promote the development of the 3Rs http://en.3rcenter.dk/symposium/symposium-2017/

3R-survey: A study of stakeholders' knowledge and experience of the 3Rs in Denmark http://en.3rcenter.dk/footer/the-3rs/the-danish-3r-survey/

Dissemination of 3R-knowledge: In collaboration with The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs, https://www.nc3rs.org.uk/) we have created the Improve your research poster. http://en.3rcenter.dk/research/improve-your-research/

Annual report: Read more in our annual reports http://en.3rcenter.dk/about-us/annual-report/

Newsletter: Stay updated on our activities through our newsletter http://en.3rcenter.dk/newsletter-subscription/

Web: www.en.3rcenter.dk

VI-244

The Danish national committee for the protection of animals used for scientific purposes – The Danish Veterinary and Food Administration, Ministry of Environment and Food of Denmark

Rasmus Nielsen

The Danish 3R-Center, Glostrup, Denmark
ranon@fvst.dk

The Danish national committee (DNC) hosts an annual network meeting for the Animal Welfare Bodies (AWBs). The meeting includes a workshop where AWB representatives exchange experience and a popular market place for presenting and sharing 3R-ideas.

https://www.foedevarestyrelsen.dk/Dyr/dyrevelfaerd/Udvalget_for_forsoegsdyr_og_alternativer/Sider/3R_tiltag_til_inspiration.aspx

In collaboration with The Danish Animal Experimentation Council the DNC has written guidelines describing standardised procedures, which are often part of applications to carry out experiments on animals.

https://www.foedevarestyrelsen.dk/Dyr/dyrevelfaerd/Dyreforsoegstilsynet/Sider/Ansoegning-og-indberetning.aspx

VI-296

Progress and challenges of the 3Rs resource platform in Korea: The way forward

Byung-in Choe[1], Gwi-hyang Lee[2,3], Moon Seok Yoon[4], Jin Soo Han[5] and Lynette Hart[6]

[1]The Catholic University of Korea, Seoul, South Korea; [2]Nicholas Cardinal Cheong Graduate School for Life, The Catholic University of Korea, Seoul, South Korea; [3]BIC Study Foundation, Seoul, South Korea; [4]Animal Protection & Welfare Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries, Gimcheon-si, South Korea; [5]Institute for the 3Rs, Konkuk University, Seoul, South Korea; [6]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, United States

bichoe@catholic.ac.kr

This year marks the 10th anniversary of proclaiming an Animal Welfare Act in Korea for the humane use of animals in science: applying the Three Rs principles of Russell and Burch and having an ethical review process prior to conducting animal experiments in research, testing, and education. It is also the 6th anniversary of establishing the Korea National Information Center for the 3Rs (hereafter referred to as KNIC3Rs). This paper summarizes the progress and challenges that have occurred during the operation of this joint collaborative effort. KNIC3Rs was initiated by the first two authors to encourage the use of methods which reduce or replace animal use or which minimize pain and distress to those animals that are used in research, testing, and education. This national platform, which was established in 2011 in collaboration with three national and five global organizations promotes awareness of moral and ethical issues surrounding animal welfare based on sound science, and facilitates the exchange of humane science and animal welfare information resources. The organizations involved are: the Institute for the 3Rs, the Division of Institutional Review Ethics of Catholic University of Korea, and the Animal, Plant and Fisheries Quarantine and Inspection Agency (all Republic of Korea); the Animalearn, the Center for Alternatives to Animal Testing, and the Animal Welfare Information Center (all USA); and the Jeanne Marchig International Center United Kingdom for Animal Welfare Education, and the Royal Society for the Prevention of Cruelty to Animals-(all UK).

This joint collaborative project encountered difficulties associated with differences in understanding and expectations, challenges to personal values and conflicts between academic and government outcomes. Yet, close collaboration among government, academia, industry and global experts is important for leveraging the power of sharing and is essential for the formulation of evidenced-based education policies. Among the barriers facing KNIC3Rs have been the slow administrative procedures as result of frequent changes in decision-making personnel, the limited budget for the operation and supporting staff provided by the Korean government agency that was primarily responsible for the platform system charged with a mission to actively engage in the production and dissemination of information to Korean researchers. Other barriers include distances from Korean facilities to KNIC3Rs, the limited space available for the resource library, and the lack of professional trainers and experts.

Aiming to develop more effective and efficient decision-making, while proactively maintaining the objectives of KNIC3Rs, the first two authors established a non-governmental organization, CITI-KOREA in 2012. This initially ran the KNIC3Rs' platform in conjunction with the government to provides a high quality, credible source of information and resources, paired with web-based research ethics education from the Collaborative Institutional Training Initiatives Program in the USA. After an initial three-year period, this private organization received government approval as a non-profit corporation, to become the first laboratory animal welfare and charitable foundation. It was renamed BIC Study in 2016. BIC Study plays a proactive role in promoting this concept with the collaborative partners and facilitates the sharing of information and resources, which will be the initial ventures in Korea. BIC Study demonstrates its commitment to integrating experts' advice and quality education, more effectively delivering resources to Korean scientists and researchers who involve human and animal research subjects. Donated books and small funds from individual and organizations; and our joint partners, UFAW, FRAME, the Joint Research Centre's European Union Reference Laboratory for Alternative to Animal Testing (EURL ECVAM), and the Scottish Accreditation Board, made progress possible for this baseline framework.

The promotion and protection of research subjects is one of the core competencies of well-educated involved personnel. Searching for the Three Rs is not a mandatory part of the training curriculum for compliance with the requirements of the Korean Animal Protection Act and other regulations. Nonetheless, training in proper searching techniques and having user-friendly systems on the practical application of the Three Rs, are essential when using animals in research and teaching. Training the next generation of researchers to foster young scientists via a guided experience, we will expand our collaborations to further develop the quality of education that provides information on laboratory animal science and alternatives in Korea.

VI-319

Improvement of DPRA for testing poorly water-soluble substances using peptide-immobilized resins containing photo-labile linkers

Kenji Usui[1], Yuuki Minamino[1], Hiroshi Miyazaki[2], Hideaki Mekata[3], Masayuki Takaishi[3] and Kunihiko Yamashita[2]

[1]Konan University, Kobe, Japan; [2]Daicel Corp., Osaka, Japan; [3]Mandom Corp., Osaka, Japan

kusui@center.konan-u.ac.jp

A direct peptide reactivity assay (DPRA) is a method to predict skin sensitization as an alternative to animal experiments. DPRA is assessed by quantifying the reactivity of chemical substances towards model synthetic peptides (Gerberick et al., 2004). Although conventional DPRA is an effective assay, there are various problems. For example, conventional DPRA hardly offers accurate measurements for poorly water-soluble substances, besides it shows low reproducibility. In order to address these limitations in DPRA, we developed a modified DPRA using peptides immobilized to resin with a photo-labile linker (Usui et al., 2016) and an unreacted peptide sequence as an internal standard. After synthesis of DPRA peptide-resin, the modified DPRA was able to assess poorly water-soluble substances. In addition, removal of unreacted chemicals provides detailed analysis of reacted peptide peaks on HPLC in this method. Our novel method would be one of the most promising alternate assay in skin sensitization testing.

References

Gerberick, G. F., Vassallo, J. D., Bailey, R. E. et al. (2004). Development of a peptide reactivity assay for screening contact allergens. *Toxicol Sci 81*, 332-343.

Usui, K., Tomizaki, K. Y. and Mihara, H. (2016). A cell microarray format: A peptide release system using a photo-cleavable linker for cell toxicity and cell uptake analysis. *Method Mol Biol 1352*, 199-210.

VI-325

A novel 3D liver microtissue model for studying steatosis *in vitro*

Sue Grepper, Fabrice Müller, Tobias Strassfeld, Simon Messner, Radina Kostadinova, Wolfgang Moritz and David Fluri

InSphero AG, Schlieren, Switzerland
sue.grepper@insphero.com

Hepatic steatosis is characterized by the accumulation of lipid droplets in the liver. We recently developed a novel 3D liver microtissue model consisting of primary human hepatocytes in co-culture with Kupffer cells, which exhibits long-term viability and liver-specific functionality. 3D human liver microtissues were treated with various concentrations of free fatty acids such as palmitate, oleate or both, and nile-red staining was performed using confocal microcopy at several time points. Oleate and palmitate alone induced time- and concentration dependent lipid accumulation, causing microvesicular and macrovesicular steatosis, respectively. The highest lipid accumulation was observed with oleate and palmitate after 7 days of treatment. The combination of both fatty acids in a physiological relevant 2:1 (oleate:palmitate) ratio resulted also in a steatotic phenotype. Palmitate and oleate induced around 3-fold induction of lipid accumulation in comparison to the vehicle control, with no reduction of cell viability. These results demonstrated that 3D liver microtissues are a suitable model for studying steatosis *in vitro*.

VI-357

Zebrafish embryo as an alternative model to predict acute oral toxicity

Lara Brito, Marize Campos Valadares and Gisele Augusto Rodrigues Oliveira

Faculty of Pharmacy, Federal University of Goiás, Goiânia, Brazil
larocabbr@gmail.com

Zebrafish embryo is a promising alternative model for traditional *in vivo* toxicity screening because it is cheaper and faster when compared to tests with mammals and complies with the 3Rs principle. We evaluated the embryotoxicity of ten substances such as acetylsalicylic acid, carbamazepine and others using Fish Embryo Toxicity (FET) test to determine LC_{50} values and compared with *in vivo* acute oral toxicity data from literature. A linear regression-model using the log-transformed of these values was generated for the prediction of LD_{50} from LC_{50} value. This model resulted in the equation Log (LD_{50}) = 0.3968 x log(LC_{50}) + 1.0652. The correlation between LC_{50} and LD_{50} was performed with 3-4-dichloroaniline and generated a LD_{50} model of 341.11 mg/kg (Category 4). Considering the oral rodents LD_{50} of 545 mg/kg (Category 4), our results confirmed that zebrafish can be an *in vivo* model for embryotoxicity and the FET represents at least a refinement in the sense of the 3Rs principle.

References

OECD (2001). Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method.
OECD (2013). Test No. 236: Fish Embryo Acute Toxicity (FET) Test.

VI-399

Procedures using cats and dogs

Marjolein Schilders-van Boxel, Jan-Bas Prins, Frank Dales, Herman Koëter, Henriëtte Bout, Coenraad Hendriksen, Wim de Leeuw, Pieter Roelfsema and Leane van Weereld

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands
Marjolein.vanboxel@rvo.nl

In its opinion "Procedures using cats and dogs", commissioned by the Minister of Agriculture (EZ), the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) has formulated specific recommendations and offers guidance on reducing the use of cats and dogs as laboratory animals without compromising the quality of research and education. The advisory report is set out under three themes, representing the fields of research where these animal species are currently used in the Netherlands: regulatory required research, education, and fundamental research. This poster will give an overview of the advisory report by the NCad.

Reference

https://english.ncadierproevenbeleid.nl/advice/documents/publications/16/11/28/ncad-opinion-procedures-using-cats-and-dogs

VI-430

The Netherlands Committee for the protection of animals used for scientific purposes (NCad) – Highlights from its first two years of existence

Marjolein Schilders-van Boxel, Herman Koëter, Jan-Bas Prins, Henriëtte Bout, Coenraad Hendriksen, Wim de Leeuw, Reineke Hameleers, Pieter Roelfsema, Frauke Ohl †, Frank Dales, Leane van Weereld, Malou Wirken, Monique Paris and Inge Toussaint

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands

Marjolein.vanboxel@rvo.nl

In the Netherlands, the government, the scientific community, industry and civil society organisations have been working hand in hand for years on the responsible use of animals in research (including scientific research) and education. The Experiments on Animals Act (Wet op de dierproeven, Wod) came into force in the Netherlands in 1977. This Act protects the welfare of laboratory animals, establishes frameworks with which procedures must comply and imposes requirements on the levels of expertise expected of those who work with laboratory animals. In late 2014, the Experiments on Animals Act was revised to bring it into compliance with the European Directive on the protection of animals used for scientific purposes (2010/63/EU). Since then, two new organisations have been active in the matter: the Central Authority for Scientific Procedures on Animals (CCD), which as an independent administrative body is the only organisation authorised to grant project licences for the performance of animal procedures in the Netherlands, and the Netherlands National Committee for the protection of animals used for scientific purposes (NCad), which has been appointed as an advisory body for the protection of animals used in research and education. These are two independent organisations, but together they contribute to improving the welfare of laboratory animals.

In order to achieve its objective, the NCad issues solicited and unsolicited opinions, fosters the development of knowledge, and builds national and international networks relating to laboratory animals, animal procedures and the Replacement, Reduction and Refinement thereof. In 2016, the NCad issued six solicited and one unsolicited opinion (against two solicted advisory reports in 2015). The committee also organised an international symposium, which helped with the further expansion of an international network. In 2015, the NCad had already taken the initiative for an informal workshop for (technical) representatives of National Committees of European Member States. When developing the opinions, the NCad was grateful to be able to draw on the expertise of many experts from both the Netherlands and beyond.

This poster will give an overview of the mission, advisory reports and other (international) activities by the NCad, its procedures for issuing opinions and plans.

Reference

https://english.ncadierproevenbeleid.nl/

VI-490

Pilot course on *in vitro* models in toxicology and students feedback tests

Francesca Caloni, Federica Danelli, Selene Festa, Francesca Genova, Valentina Granata, Francesca Grassi Scalvini, Silvia Morelli, Chiara Pelizzoni, Lucia Schiavone and Virginia Bassi

Dipartimento di Medicina Veterinaria (DIMEVET), Università degli Studi di Milano, Milan, Italy

francesca.caloni@unimi.it

A new course on *in vitro* toxicology started with the AY 2015/2016, first semester, in the University of Milan, in the second year of the master degree of Veterinary Biotechnology Sciences, entitled "Toxicology and *in vitro* models". The course is 3 credits, 30 hours, inclusive of 6 hours of lectures and 24 hours of practice. The purpose of the course is to provide tools and information, on *in vitro* tests and models for toxicological studies, with a particular attention on the emerging techniques. The didactic material, inclusive of photographic material of the lab-activity related to the topic, is up-loaded on a dedicated online platform (www. ariel.unimi.it), available for the students, up-dated from time to time by the teacher of the course. Through a meeting with experts, an approach of comparison between *in vitro* and *in silico* data was also addressed.

At the end of the course a feedback of students was also required, by filling a questionnaire. They found very important to be informed about this topic, as *in vitro* techniques represent a useful alternative approach that has gained prominence in recent years, also in an integrated testing strategy and they believe that the knowledge of this discipline is fundamental for their career.

Reference

Sachana, M., Theodoridis, A., Cortinovis, C. et al. (2014). Student perspectives on the use of alternative methods for teaching in veterinary faculties. *Altern Lab Anim 42*, 223-233.

VI-518

Campaigning for replacement alternatives in Iran

Ramak Roshanaie[1,2], Nick Jukes[3] and Shahabeddin Safi[2]

[1]IAVA – InterNiche Iran, Tehran, Iran; [2]IAVA – Faculty of Veterinary Medicine, Science & Research, Islamic Azad University, Tehran, Iran; [3]InterNICHE, Leicester, United Kingdom

ramak_r_m@yahoo.com

The Iranian Anti-Vivisection Association (IAVA) is an academic organisation founded in 2010 that works to introduce humane alternatives and replace harmful animal use in education and training. As an InterNICHE Partner, the organisation collaborates with teachers, students and university administrations, and lobbies government ministries for change. Seminars and exhibitions have been held at veterinary congresses, and some alternatives distributed nationwide. Despite the challenging environment, successes have already been achieved. The network has grown, and replacement facilitated in a number of anatomy, pathology, physiology, pharmacology, toxicology and surgery practical classes. IAVA also promotes alternatives in research and testing. Critiquing animal experimentation, informing people about alternatives, and demonstrating that full replacement is achievable, is happening for the first time in Iran.

IAVA jointly won the 2016 Lush Prize for Training.

VI-528

A new approach to advancing the 3Rs: The North American 3Rs Collaborative (NA3RsC)

Natalie Bratcher[1], Norm Peterson[2], Deb Curry[3], Szczepan Baran[4], Mary Ann Vasbinder[5] and Marilyn Brown[6]

[1]Office of Animal Welfare & Compliance, AbbVie, North Chicago, IL, United States; [2]Veterinary Services, Medimmune, Gaithersburg, MD, United States; [3]Event Management, Charles River, Wilmington, MA, United States; [4]Animal Welfare Compliance Training, Novartis, Cambridge, MA, United States; [5]Office of Animal Welfare, Ethics and Strategy, GlaxoSmithKline, King of Prussia, PA, United States; [6]Global Animal Welfare, Charles River, Cambridge, MA, United States

natalie.bratcher@abbvie.com

Several groups across North America include 3Rs advancement in their objectives. Recognizing a lasting need to foster a more centralized approach with improved communication, collaboration, reporting and implementation of the 3Rs, several members of the research community founded the North American 3Rs Collaborative (NA3RsC). The mission of the NA3RsC is to advance the education and science of the 3Rs – replacing, reducing and refining the use of animals in research. Members include academia, industry, and government. In our first year, the NA3RsC elected a Board of Directors, approved its by-laws, and gained 501(c) 3 status. The NA3RsC is sponsoring presentations at WC10, AALAS and is co-sponsoring the 3Rs Sharing Conference. Our online collaborative space, the Virtual Education Community (VEC), is one of our most value-added tools. The VEC includes a theater to host live symposia, a community pavilion for 3Rs groups, an area for 3Rs-related vendors, a resource hub, and a platform for 3Rs-related discussions. The NA3RsC looks forward to working collaboratively for broad reaching impact and to set a new precedent for 3Rs culture in North America.

VI-532

Animal experimentation in Serbia: National surveys and a retrospective analysis

Katarina Novaković

InterNICHE Serbia and ORCA Belgrade, Belgrade, Serbia
kahabg@gmail.com

In 2006 and 2014, surveys of all universities and biomedical institutes in Serbia were conducted to gather data on animal use in education, research and testing. The survey questions referred to the number and species of animals, the purpose of the animal use, and other topics. The questions were consistent between both surveys, and in both cases the law on access to information of public importance was used. In 2009, the country's first Animal Protection Act was passed. This poster will present data from 2005 and 2013, to explore the situation before and after the Act. The number and species of animals used for dissection and experiments in education, research and testing will be presented. An analysis of the changes in the results of the two surveys will be given, demonstrating where replacement is most evident, and where the 3Rs have failed to be implemented. In the absence of official data and statistical analysis, this is the first retrospective analysis for the country.

Reference

Taylor, K., Gordon, N., Langley, G. and Higgins, W. (2008). Estimates for worldwide laboratory animal use in 2005. *Altern Lab Anim 36*, 327-342.

VI-535

Protective effect of natural plant extracts on UV-induced fin damage in zebrafish

Jie Liu[1], Fengjuan Wu[1], Chen Zhang[1], Shihua Yuan[2], Weidong Wang[3], Kangdi Zheng[4] and Weijie Mei[4]

[1]Infinitus (China) Company Ltd, Guangzhou, People's Republic of China; [2]Guangzhou Logicos Biotech Co., Ltd, Guangzhou, People's Republic of China; [3]Guangdong Food and Cosmetic Testing Centre, Guangdong, People's Republic of China; [4]School of Pharmacy, Guangdong Pharmaceutical University, Guangdong, People's Republic of China

my_lj2001@163.com

In zebrafish, UV exposure leads to fin malformation phenotypes including fin reduction or absence (Tsai et al., 2012; Cheng et al., 2014; Chen et al. 2011). We used ultraviolet (UV)-induced fin damage in zebrafish larvae as a system for evaluating the chemopreventive potential of extracts of four species of natural plant, including *Nelumbium speciosum* flower (Di and Fonseca, 2005), *Sophora japonica* bud (Paniwnyk et al., 2001), *Crocus sativus* flower (Baba et al., 2015), and *Solanum lycopersicum* (Tomato) fruit (Grozeva et al., 2013), by recording fin morphological changes. The results showed that all of zebrafish larvae of control group (- UV) and UV + natural plant extracts group displayed normal fins, but zebrafish larvae exposed to UV showed higher incidence of fin abnormalities, including fin absence or reduction. Moreover, the area of zebrafish fins of the mock control group (- UV) and UV + natural plant extracts group were similar, and were larger compared with that in the UV only group (+ UV). Inconclusion, this natural plant extracts have potential protective effects on UVB-induced fin damage in zebrafish larvae, which might be useful in pharmaceutical and cosmetic formulations.

References

Baba, S. A., Malik, A. H., Wani Z. A. et al. (2015). Phytochemical analysis and antioxidant activity of different tissue types of crocus sativus, and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. *S Afr J Bot 99*, 80-87.

Cheng, C. C., Chou, C. Y., Chang, Y. C. et al. (2014). Protective role of comfrey leave extracts on UV-induced zebrafish fin damage. *J Toxicol Pathol 27*, 115-121.

Chen, Y. H., Wen, C. C., Lin, C. Y. et al. (2011). UV-induced fin damage in zebrafish as a system for evaluating the chemopreventive potential of broccoli and cauliflower extracts. *Toxicol Mech Meth 21*, 63-69.

Di, M. V. and Fonseca, M. J. (2005). Assays of physical stability and antioxidant activity of a topical formulation added with different plant extracts. *J Pharm Biomed Anal 37*, 287-295.

Grozeva, S., Atanasova, A. and Denev, P. (2013). Polyphenolic constituents, antioxidant activity and mineral content of very small and small-fruited tomato (solanum lycopersicum L.) genotypes. *Agrochimica 57*, 337-346.

Paniwnyk, L., Beaufoy, E., Lorimer, J. P. and Mason, T. J. (2001). The extraction of rutin from flower buds of sophora japonica. *Ultrason Sonochem 8*, 299-301.

Tsai, I. T., Yang, Z. S., Lin, Z. Y. et al. (2012). Flavone is efficient to protect zebrafish fins fromUV-induced damage. *Drug Chem Toxicol 35*, 341-346.

VI-554

Introducing the Swedish 3Rs Center

Torsten Jakobsson, Per E. Ljung, Cecilia Bornestaf, Karen Erlbacher, Anders Elmström and Anna Heimersson
Swedish Board of Agriculture, Jönköping, Sweden
torsten.jakobsson@jordbruksverket.se

The Swedish parliament decided in December 2016 to finance a Swedish 3Rs Center for the coming four years. The center is the acting body of the Swedish committee for the protection of animals used for scientific purposes. This committee shall advise concerned authorities and animal welfare bodies on matters dealing with the care and use of animals in procedures and ensure sharing of best practice. As acting body, the 3Rs Center's main purpose is to promote and coordinate the work with replacement, reduction and refinement of animal use for scientific purposes in Sweden. Counselling, collection and dissemination of information on these topics are main tasks. The Swedish 3Rs Center is located at the Swedish Board of Agriculture and will have its official opening in November 2017.

VI-564

Training and competency of animal users, carers and animal ethics committee members

Amanda Fernie
Griffith University, Nathan, Australia
a.fernie@hotmail.com

The Australian code for the care and use of animals for scientific purposes 8th edition (2013) requires that institutions "ensure that all people involved in the care and use of animals understand their responsibilities and the requirements of the Code, are competent in the procedures they perform or are under the direct supervision of a person who is competent to perform the procedures".

This presentation will describe the training and competency framework in place at Griffith University and share some findings from an analysis of the aggregated test results for our theoretical training modules.

VI-581

Introducing the 3R concepts into pre-college education

Nicole Green[1], Gertrude-Emilia Costin[2], Adele Miller[2], Sue Leary[3] and Katherine Lewis[1]
[1]Education, Animalearn, Jenkintown, PA, United States; [2]Institute for In Vitro Sciences, Inc., Gaithersburg, MD, United States; [3]Alternatives Research & Development Foundation, Jenkintown, PA, United States
ngreen@animalearn.org

This poster will provide a snapshot of precollege teachers (46) within the U.S. gauging their knowledge and familiarity of the 3Rs and non-animal testing methods (i.e. *in vitro* methods), initiative to address the topic in the classroom, as well as assessment of their students' level of interest in the topic.

While recognizing the ethical considerations related to animal experimentation, the current generation of teachers and students is also eager to understand the relevance, reliability and reproducibility of *in vitro* methods as the modern wave of technologies in toxicology and possible replacement of animals use for testing purposes.

Our data indicates an education field eager to learn about new concepts that might impact our daily activities in an ethical way and to get up to speed with advances in science.

VI-657

K-12 teacher attitudes on dissection alternatives

Nicole Green and Katherine Lewis
Education, Animalearn, Jenkintown, PA, United States
ngreen@animalearn.org

Our study is based on surveys of K-12 science teachers taken at science teacher conferences in the U.S. over a 4-year period. We found that over half of the respondents, 50.4%, never tried alternatives; 33.9% tried alternatives to dissection and reported a positive experience; 10% tried alternatives and reported a negative experience; and 5.7% had either no response or reported other comments.

Of the teachers who had a positive experience, over 50% indicated that they would like to try other alternatives to ensure that they choose the best replacement available, while 32% of the teachers responded that their school lacked funding for alternatives. Our survey data suggests that teachers need increased exposure and access to alternatives to dissection. We include recommendations for enhancing current efforts.

VI-679

U.S. public opinion on the use of animals for medical training: Analysis of a randomized 2016 telephone survey

Ryan Merkley[1] *and Ari Joffe*[2]

[1]Research and Regulatory Affairs, Physicians Committee for Responsible Medicine, Washington, DC, United States; [2]Department of Pediatrics, University of Alberta, Edmonton, AB, Canada
rmerkley@pcrm.org

The U.S. public appears to be increasingly concerned about the use of animals in laboratory experimentation (Riffkin, 2015; Goodman et al., 2012; Funk and Rainie, 2015). Yet most surveys have asked broad questions about the use of animals in "research" or "testing." While the public is familiar with those terms, the reality of laboratory animal use is far more diverse. We commissioned a national survey aimed at understanding public perceptions of a specific area of laboratory animal use – medical training. A random telephone survey of 1,011 U.S. residents was performed by ORC International in March 2016. For all questions about training medical students, physicians, emergency physicians, paramedics, and pediatricians, the large majority of respondents (82-83%) agreed that non-animal methods should be used. The large majority of respondents (83-84%) agreed they want their doctor trained using non-animal methods. A majority (66-67%) agreed that using live animals to train medical professionals "is morally wrong or unethical."

References

Funk, C. and Rainie, L. (2015). Pew Research Center. Chapter 7: Opinion About the Use of Animals in Research. http://www.pewinternet.org/2015/07/01/chapter-7-opinion-about-the-use-of-animals-in-research/

Goodman, J. et al. (2012). Mounting Opposition to Vivisection. *Contexts 11*, 68. http://journals.sagepub.com/doi/abs/10.1177/1536504212446466

Riffkin R. Gallup, Inc. (2015). "In U.S., More Say Animals Should Have Same Rights as People." http://www.gallup.com/poll/183275/say-animals-rights-people.aspx

VI-686

Services for animal research around the 3Rs at Comparative Medicine, Karolinska Institutet, Sweden

Viveka Hillegaart, Rafael Frias, Johannes Wilbertz and Brun Ulfhake

Comparative Medicine, Karolinska Institutet, Stockholm, Sweden
viveka.hillegaart@ki.se

Comparative Medicine (CM) was founded in 2010 to become the major responsible infrastructure at Karolinska Institutet for the care and use of animals in preclinical research and as models of human diseases. CM's missions include the operation and coordination of all laboratory animal resources at KI, and development of this infrastructure to the level of excellence and in coherence with the demands of the research community. CM initiatives are governed by evidence-base practice and the 3Rs; and actively promotes animal welfare and that all research conducted on animals is of the highest quality. CM provides education in laboratory animal science and supervise adherence to national and EU laws and regulations. CM is also a major player in Sweden, taking an active role in LAS matters on the national and EU level.

Animal used for research on human diseases at KI range from cyclostomata to non-human primates (NHP), and the dominating species is the mouse (> 90%, year 2015). The use of animals and the procedures and analyses conducted have developed rapidly in the past years, and these changes pose challenges both for the design and the operation of new animal facilities. Most animals used today are genetically engineered models often produced and bred in-house, thus only about 3 out of 5 cage-slots holding mice are used for animals in experiment; and procedures are conducted with more and more advanced equipment demanding more laboratory space. The projected need up until 2023 was set to 49,000 holding units and 150 laboratories on campus Solna. The lab animal facilities are planned to include sophisticated analysis with e.g. high-resolution MRI and PET (including use of short-lived ligands), radiation source(s), advanced behavioral and system physiology analyses, housing under different climate and biosafety conditions, and more.

Reference

Annual Report 2016, Comparative Medicine, Karolinska Institute, Stockholm 2017.

VI-689

Recombinant antibodies: Replacement in action

Charu Chandrasekera

Research Policy, Physicians Committee for Responsible Medicine, Washington, DC, United States
cpchandr@gmail.com

The majority of antibodies used for research are currently generated *in vivo* using various animal species. These antibodies often lack specificity and contribute to research irreproducibility. In addition to performance issues, animal antibody production methods are inherently cruel. The purpose of this study was to generate experimental evidence to promote the widespread acceptance and adoption of recombinant antibodies for basic research. In Phase I, we have generated a recombinant antibody against the DYKDDDDK epitope tag widely used for recombinant protein expression. Side-by-side comparisons against commercial DDK antibodies, including an ascites antibody, indicate that our recombinant antibody is functionally comparable to the animal-derived clones in various immunological applications. Recombinant antibodies can obviate the need for animal-derived antibodies, creating opportunity for industry-wide acceptance and adoption of this technology for research antibody production.

Theme VII – Translation

Coordinators
Raymond R. Tice, NIEHS, Division of the National Toxicology Program, Durham, NC, United States
Tuula Heinonen, FICAM, University of Tampere, Department of Medicine, Tampere, Finland

Oral Presentations

Session VII-1: Biomarkers

Co-Chairs
Elizabeth Walker, Critical Path Institute, Tuscon, AZ, United States
Frank Sistare, Merck & Co., Inc., West Point, PA, United States

VII-1-829

Regulatory qualification of biomarkers: Determining the level of evidence necessary for use

Elizabeth Walker
Critical Path Institute, Tucson, AZ, United States
ewalker@c-path.org

A number of collaborations have been launched thru scientific consortia to advance the understanding and use of translational biomarkers in facilitating drug development. For full biomarker utility and productive collaborations, clear regulatory expectations for their use in decision making are needed. Recently a multi-stakeholder effort including government, industry and academia convened to develop an evidentiary criteria (EC) framework for use in biomarker qualification. This biomarker qualification framework is intended for broad application across multiple biomarker categories and contexts of use, and should assist stakeholders in determining how much and what specific types of evidence and data are necessary to put biomarkers into routine use in drug development. Examples of initiatives underway to advance regulatory acceptance of translational biomarkers will be provided.

VII-1-754

Regulatory qualification and applications of new translational kidney safety biomarkers in drug development

Frank Sistare
Merck & Co., Inc., West Point, PA, United States
frank_sistare@merck.com

New translational kidney safety biomarkers are now enabling drug development by: 1) enhancing safety monitoring of patients in early clinical trials for toxicities seen in animal studies that are of questionable human relevance, 2) providing early insights to pathogenic mechanisms leading to drug induced kidney injury in early drug development toxicology studies, and 3) assisting early compound selection and animal de-risking studies that reduce the probability of later attrition due to kidney toxicity. Several consortia including the FNIH Biomarker Consortium Kidney Safety Biomarker Project Team are presently collaborating on additional progressive qualification efforts in partnership with regulatory agencies and academia to further advance the qualification of novel translational renal safety biomarkers using samples from retrospective observational clinical studies in a learning mode, and prospectively designed confirmatory clinical studies.

VII-1-787

Carcinogenicity testing of new pharmaceuticals: A weight of evidence approach

Markku Pasanen

UEF, School of Pharmacy, Kuopio, Finland
markku.pasanen@uef.fi

The usefulness of carcinogenicity testing of every new pharmaceutical, in terms of human safety, has been debated for several years. In the ICHS1 work plan partners form regulatory and industry sides, in a prospective manner, try to resolve the relevance of the 2 year rodent bioassay in terms of human safety. The weight of evidence depends on all non-clinical signals, including *in silico*, *in vitro*, repeated dose toxicity studies in rodents and non-rodents which may contribute to development of malignancy, cell proliferation and uncontrolled cell growth. Important signals in focus are for instance genotoxicity, hormonal perturbations, immunosuppression, generation of reactive metabolites and metabolite profiles between non-clinical species and man. To serve this approach, Sponsors are also encouraged to submit "omics" and biomarker-based novel data which could guide and convince all parties in selecting proper testing strategies to demonstrate carcinogenic potential of their compound.

VII-1-828

Glutamate dehydrogenase: An emerging biomarker of liver injury that enables drug development and improves medical care

Jiri Aubrecht

Safety Biomarker Laboratories, Pfizer Global Research and Development, Groton, CT, United States
jaubrecht@pfizer.com

The hypertransaminasemia due to underlying muscle damage creates a significant diagnostic challenge. Thus the development of liver specific biomarkers of hepatocyte injury is essential. We investigated the potential of glutamate dehydrogenase (GLDH), a liver mitochondrial enzyme, to serve as a liver specific biomarker of hepatocellular injury. We demonstrated that serum GLDH successfully detects liver injury in patients with underlying muscle diseases and in contrast to ALT is independent of metabolic effects. Although additional confirmatory studies are needed, our data indicate that GLDH might be a better measure of liver health than currently used serum gold standard transaminases.

VII-1-703

Gene environment interaction for autism in a brain organoid model

Lena Smirnova[1], Xiali Zhong[1], David Pamies[1], Helena Hogberg[1], Herbert Lachman[2] and Thomas Hartung[1]

[1]CAAT, Johns Hopkins University, Baltimore, MD, United States; [2]Albert Einstein College of Medicine, Baltimore, MD, United States
lsmirno1@jhu.edu

The strong increase of autism in recent years cannot be explained solely by genetics but rather by gene environment interactions. A combination of three technologies (iPSC, CRISPR-CAS9 gene editing and 3D culture technologies) allows to produce an *in vitro* 3D brain model with autistic genetic background. Mutation in the CHD8 gene is one of the major genetic risk factors for autism. A heterozygous $CHD8^{+/-}$ knockout iPSC cell line was generated using CRISPR-CAS9 technology. $CHD8^{+/-}$ iPSC and control iPSC from the same donor ($CHD8^{+/+}$) were neurally differentiated in 3D and exposed to chlorpyrifos (CPF) for 24 h. $CHD8^{+/-}$ brain organoids were more sensitive to CPF than $CHD8^{+/+}$ on the level of cell viability, gene expression, acetylcholinesterase inhibition, synaptogenesis, and neurite outgrowth. Thus, there is a potential synergy of two autism risk factors, i.e., CHD8 mutation and CPF. Further multi-omics approaches will allow to identify molecular mechanisms of this potential synergy.

References

Pamies, D., Barreras, P., Block, K. et al. (2017). A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity. *ALTEX 34*, 362-376. doi:10.14573/altex.1609122

Wang, P., Lin, M., Pedrosa, E. et al. (2015). CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Molecular Autism 6*, 1. doi:10.1186/s13229-015-0048-6

Session VII-2: Relevant Disease Models

Co-Chairs
Thomas Hartung, CAAT, Johns Hopkins University, Baltimore, MD, United States
Tuula Heinonen, FICAM, University of Tampere, Tampere, Finland

VII-2-830

From microphysiological to micropathophysiological systems

Thomas Hartung[1,2]

[1]Centers for Alternatives to Animal Testing in the US and Europe, Johns Hopkins University, Baltimore, MD, United States;
[2]University of Konstanz, Konstanz, Germany
thartun1@jhu.edu

Enormous progress especially in the generation of human tissue equivalents from stem cells and more organotypic culture approaches have allowed to recapitulate some of their physiological properties, i.e. microphysiological systems (MPS). Increasingly, the field is now moving to disease models to ultimately attempt interventions. Toxic effects of various agents appear to be the easiest to model, though the limitations of xenobiotic metabolism prevail also in MPS. Infections with viruses, bacteria and others are relatively straight-forward, but the common lack of an immune systems represents a major drawback. The introduction of cancer cells into organoids represents another important class of disease models, which allow to test chemotherapies with the expectation that the grafted tumors behave more clinically relevant within a healthy tissue and that personalized "precision medicine" results can be obtained for a given patient's cancer. As most MPS are derived now from stem cells, especially developmental can be studied, e.g. using stem cells from donors with specific genetic defects. More chronic and degenerative disorders are similarly addressed using patient's stem cells, but the time required to build a disease phenotype can often not be met in culture, though in some cases hallmarks of the disease were achieved rather fast. For most chronic diseases, however, there is an interplay of genetic and environmental (exposure) components. While these models actually allow for the first time to study such gene x environment interactions, the endless number of combinations still represents an enormous challenge. The expectations are high, though unproven, that such disease models will complement and replace animal models for drug development.

The lecture uses the development of a human mini-brain to illustrate these developments and their challenges.

VII-2-604

A human heart-liver platform to study drug metabolism and toxicity

Anne Riu[1], *Carlota Oleaga*[2], *Gregg Legters*[2], *Lee Kumanchik*[2], *Vivien Platt*[2], *Richard Bridges*[2], *Candace Martin*[2], *Max Jackson*[2], *Christopher McAleer*[2], *Christopher Long*[2], *Jessica Langer*[3], *Reine Note*[1], *James Hickman*[2], *Silvia Teissier*[1], *José Cotovio*[1] *and Lionel Breton*[1]

[1]L'Oréal Research and Innovation, Aulnay sous Bois, France;
[2]University of Central Florida, Orlando, FL, United States;
[3]L'Oréal Research and Innovation, Clark, NJ, United States
ARIU@rd.loreal.com

Regulation of cosmetic testing has spurred efforts to develop new methods for systemic toxicity but *in vitro* assays do not fully represent physiology, often lacking xenobiotic metabolism. Thus, we have developed a human heart-liver system to study metabolism-dependent toxicity combining fully functional cardiac and hepatic modules maintained in serum-free medium, under flow, for 14 days. Changes in cardiac beat frequency and contractile force, and hepatic enzyme, albumin and urea profiles were studied. Cyclophosphamide and terfenadine, used for system validation, represent both case scenarios: a non-cardiotoxic pre-drug that converts into a cardiotoxic metabolite, and the opposite. Integration of metabolic function in toxicology models can improve adverse effects prediction. Our system enables functional readout of cardio- and hepatotoxicity for acute and chronic dosing of drugs and metabolites.

VII-2-715

The assessment of angiogenesis/ vasculogenesis in the context of developmental toxicity

Tuula Heinonen and Tarja Toimela

Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
tuula.heinonen@staff.uta.fi

Vascular disruption is one of the principal teratogenic mechanisms suspected to be associated with medication (van Gelder et al., 2014). FICAM has developed a human cell based vasculogenesis/angiogenesis test. The test has been thoroughly characterized on structural, gene expression and functional levels and validated: reproducibility, repeatability and relevance were proven.

The relevance to predict inhibition of tubule formation in man was shown with drugs with published human data resulting in a good concordance (EC_{50} vs human plasma C_{max} at therapeutic dose, Pearson R = 0.6921). The performance was further evaluated using randomly selected set of chemicals with teratogenic potential (https://www.purdue.edu/ehps/rem/ih/terat.htm). The results showed that 15 of the 20 randomly selected teratogens were vasculogenesis/angiogenesis inhibitors (Toimela et al., 2016) and confirmed the crucial role of inhibition of vasculature in teratogenesis. The pathway analysis is ongoing.

References

Toimela, T., Huttala, O., Sabell, E. et al. (2016). Intra-laboratory validated human cell-based in vitro vasculogenesis/angiogenesis test with serum-free medium. *Reprod Toxicol 70*, 116-125.

van Gelder, M. M., van Rooij, I. A., de Jong-van den Berg, L. T. and Roeleveld, N. (2014). Teratogenic mechanisms associated with prenatal medication exposure. *Therapie 69*, 13-24.

VII-2-237

Predicting human drug toxicity and safety via animal tests: Can any one species predict drug toxicity in any other, and do monkeys help?

Jarrod Bailey[1], Michael Balls[2] and Michelle Thew[1]

[1]Cruelty Free International, London, United Kingdom; [2]University of Nottingham, Nottingham, United Kingdom
jarrod.bailey@crueltyfreeinternational.org

Drug attrition is at an all-time high of 95%, yet development still rests squarely on animal tests. In the absence of robust evidence to support this, we have examined claims that animal data contribute significant evidential weight to the toxicity (or lack of toxicity) of new drugs in humans. We show that the absence of toxicity in dogs, rats, mice, rabbits, and even monkeys, provides essentially no insight into the likelihood of a similar absence of toxicity – or "safety" – in humans. This is the critical factor for the progression of new drugs into clinical trials. The probability of a new drug not being toxic in humans is increased from, say, 70% to just 70.4% by a negative test result in monkeys, or just 74% on average by any of the species. The animal tests therefore provide essentially no additional confidence in human outcome, but at great ethical and financial cost. This should have widespread implications for the pharmaceutical industry and regulators.

Reference

Bailey, J., Thew, M. and Balls, M. (2015). Predicting human drug toxicity and safety via animal tests: Can any one species predict drug toxicity in any other, and do monkeys help? *Altern Lab Anim 43*, 393-403.

VII-2-674

Mouse sepsis studies: Attending to the data

Emily Trunnell[1] and Alka Chandna[2]

[1]Laboratory Investigations Department, People for the Ethical Treatment of Animals, Norfolk, VA, United States; [2]Laboratory Investigations Department, People for the Ethical Treatment of Animals, Washington, DC, United States
EmilyT@peta.org

A 2013 landmark study published in PNAS raised substantial concerns about the relevance of mouse sepsis experiments to answer questions about sepsis in humans. The analysis concluded that responses in mice following acute inflammatory stressors such as sepsis were "close to random in matching their human counterparts" and supported the "higher priority for translational medical research to focus on the more complex human conditions rather than relying on mouse models." National Institutes of Health (NIH) Director Francis Collins commented on these findings, lamenting that over "150 drugs … successfully treated this condition in mice," only to fail in human trials. However, NIH continues to fund hundreds of mouse sepsis experiments in projects that consume tens of millions of dollars and tens of thousands of mice. We consider gaps in the oversight system that permit critical data to be overlooked in evaluating experiments of questionable value.

References

Collins, F. (2013). Of mice, men, and medicine. https://directorsblog.nih.gov/2013/02/19/of-mice-men-and-medicine/

Fink, M. P. (2014). Animal models of sepsis. *Virulence 5*, 143-153.

Seok, J., Warren, H. S., Cuenca, A. G. et al. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A 110*, 3507-3512. doi:10.1073/pnas.1222878110

VII-2-573

Ex vivo micrometastasis model using fresh human lung tissue and patient-derived, disseminated melanoma cells

Sebastian Konzok[1], Susann Dehmel[1], Christian Werno[2], Peter Braubach[3], Gregor Warnecke[3], Patrick Zardo[3], Danny Jonigk[3], Olaf Pfennig[4], Hans-Gerd Fieguth[4], Bernhard Polzer[2], Kathrin Weidele[2], Christoph Klein[2], Armin Braun[1] and Katherina Sewald[1]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [2]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Regensburg, Germany; [3]Medical School Hannover, Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [4]KRH Clinics, Hannover, Germany

sebastian.konzok@item.fraunhofer.de

Metastasis is the main cause of death in cancer and characterized by high cellular heterogeneity and genetic disparity in regards to the primary tumor. Currently, this complex process can only partly be reflected in *in vitro* models due to lacking human microenvironment. Metastatic tumor growth is modulated within its natural microenvironment by adding disseminated melanoma cells to fresh human lung tissue.

Cancer cells integrate into the lung tissue and increase 6-fold within the first day of culture. Cells decrease until day 3 but recover after 5 days (5.4-fold increase). Macrophages infiltrate melanoma accumulations (19-fold increase) and interact with selected cells. Treatment with Vemurafenib (50 μM) on tissue invaded by V600E melanoma decreases cancer cell number by 71% while non-mutated cells are unaffected.

Here we mimic cancer cell proliferation, growth and interactions in human lung tissue. Treatment with established drugs showed patient-specific reduction of cancer cell growth.

VII-2-229

Hepatic cells derived from adult human skin stem cells as an *in vitro* model to study non-alcoholic fatty liver disease (NAFLD)

Vera Rogiers, Joost Boeckmans, Alessandra Natale, Karolien Buyl, Joery De Kock, Tamara Vanhaecke and Robim M. Rodrigues

Vrije Universiteit Brussels, Brussels, Belgium

vrogiers@vub.ac.be

NAFLD ranges from reversible steatosis to life threatening non-alcoholic steatohepatitis. No predictive, human-based *in vitro* system exists that accurately represents the molecular mechanisms involved in the progression of NAFLD.

Stem cells, isolated from human skin, were differentiated into hepatic cells upon sequential exposure to hepatic growth factors that play a role during liver development. When exposed to steatogenic compounds (tetracycline, valproate and insulin), lipids accumulated intracellularly. We investigated the molecular mechanisms involved by the modulation of the expression of key genes and compared the results with exposed HepaRG™ cells. In our cells an increased *de novo* lipogenesis (*SCD1* upregulation), a decreased fatty acid β-oxidation (*ACADSB* and *CPT-1* downregulation) and a decreased VLDL secretion (*APOB* downregulation) was found, which makes them of interest as preclinical model. In exposed HepaRG™ cells no induction of *de novo* lipogenesis was observed.

Session VII-3: Best ES/IPCS Practices

Co-Chairs
Blake Anson, Cellular Dynamics International, Madison, WI, United States
Xianmin Zeng, Buck Institute for Research on Aging, Novato, CA, United States

VII-3-740

Maximizing the potential of human iPSC models through rigorous processes and validations

Blake Anson
Cellular Dynamics International, Madison, WI, United States
banson@cellulardynamics.com

Human induced pluripotent stem cell (iPSC)-based technology has the potential to bring relevant human biology to the lab bench in a variety of new and previously unattainable formats. However, realizing the maximal utility of such models requires careful attention to the creation and maintenance of the parental iPSC line, differentiation into particular model cell types, and a clear and extensive validation of their benefits and limitations. This presentation will discuss the changing state of the art in somatic cell reprogramming, definitions and tests of pluripotency, guiding principles of cellular differentiation, and real world examples where iPSC-derived cardiomyocytes are replacing animal-based models in safety and toxicity testing, example areas (and work-arounds) of functional limitations, and how a highly quality controlled neuronal differentiation process has led to replacement of rodent models in batch release of botulinum-toxin.

VII-3-618

Leveraging novel technologies for human iPSC-based screening

Xianmin Zeng
Buck Institute for Research on Aging, Novato, CA, United States
xzeng@xcellscience.com

Human induced pluripotent stem cell (iPSC) technology offers the benefits of a cell line coupled with the advantage of using human primary cells. We have developed a panel of iPSC lines for neurotoxicity assays and disease modeling. These include: 1) control lines, 2) patient-specific lines, 3) lineage-specific knock-in reporters, 4) isogenic controls of single and double knock-outs. We have also established scalable protocols for generating differentiated cells in an assay ready format. I will discuss the utility of these lines for neurotoxicity assays including assays to determine the specificity of different neural cell types for a small range of chemicals and drugs from the Tox21 library, as well as for neuroprotective assays with dopaminergic neurons.

VII-3-721

Functional screening assays with iPSC and iPSC derived neural derivatives

Mahendra Rao
Regenerative Medicine, XCell Science, Novato, CA, United States
mrao@xcellscience.com

Pluripotent stem cells (PSCs) possess the special characteristic of being able to develop into any cell type in the body. This unique trait and the ability to derive PSCs from patients make this cell type an ideal starting point to identify toxicologic effects of commonly used compounds Therapeutic options for neurodegenerative disorders could be particularly improved by a PSC-based strategy to generate neural cells for use in drug screens. We have developed an efficient protocol to generate neural stem cells (NSCs) from PSCs and in turn convert these NSCs to very pure populations of neurons and astrocytes. Gene expression analyses indicate that the neurons and astrocytes express genes that are part of functional pathways known to be active in these cells. In this presentation, I will describe how we have developed standardized protocols to generate iPSC derived neural cells from normal, patient specific and gene engineered iPSC lines. I will discuss the importance of comparing results between human and rodent cells and the advantages and limitations of these cells.

VII-3-350

Metabolically functional stem cell-derived hepatocytes

Yaakov Nahmias
Hebrew University, Jerusalem, Israel
ynahmias@gmail.com

Hepatocytes play a critical role in drug metabolism, but their utilization is limited by scarcity and batch-to-batch variability. Regretfully, differentiation of stem cell-derived hepatocytes produces fetal cells with limited metabolic capacity. Here we show that the postnatal microbiome drives the metabolic maturation of hepatocytes through a PXR and PPARα mediated mechanism. We show a 4-fold expansion, with 83 ±4% of the cells positive for albumin and HNF4α after 18 days, permitting high-content screening in 96-well format. We show CYP450 induction in response to PXR, CAR, and AhR agonists, and a shift from glycolytic to oxidative phenotype marked by robust mitochondrial maturation. Analysis of 12 compounds showed a 0.94 correlation between TC50 values obtained in our stem cell-derived hepatocytes and primary cells. Finally, stem cells-derived hepatocytes demonstrated steatosis, apoptosis, and cholestasis confirming the ability of our system to produce predictive results.

Reference

Avior, Y., Levy, G., Zimerman, M. D. et al. (2015). Microbial-derived lithocholic acid and vitamin K2 drive the metabolic maturation of pluripotent stem cells derived hepatocytes. *Hepatology 2*, 265-278.

VII-3-776

Read-across and grouping of complex substances using bioactivity data from human induced pluripotent stem cell (iPSC)-derived models

Ivan Rusyn and Fabian Grimm

Texas A&M University, College Station, TX, United States
irusyn@tamu.edu

Complex chemical products and mixtures are difficult to characterize with respect to their chemical composition. However, these substances can be screened *in vitro* and the information on global similarities in their bioactivity can be used for grouping and read-across. This presentation will describe the use of a suite of IPSC-based human *in vitro* models and petroleum substances as a case study. For phenotypic screening, we exposed induced pluripotent stem cell-derived cardiomyocytes, hepatocytes, neurons, endothelial cells and macrophages to DMSO-soluble extracts of petroleum substances from distinct product groups. Concentration-response profiling and high-content imaging for bioactivity, as well as targeted transcriptomics revealed distinct signatures for groups of petroleum substances. Data integration showed that bioactivity profiling resulted in clustering of these substances in a manner similar to the manufacturing process-based categories. Moreover, we observed a high degree of correlation between bioactivity profiles and physico-chemical properties, as well as improved groupings when chemical and biological data were combined. Altogether, we demonstrate how novel *in vitro* screening approaches can be effectively utilized in combination with physico-chemical characteristics to categorize complex substances and mixtures, thereby indicating the utility of the novel data streams in regulatory submissions with respect to building confidence in read-across.

VII-3-708

Promise and pitfalls of induced pluripotent stem cells: Learning from past mistakes

Ann Lam[1,2] and Elan Ohayon[2]

[1]Physicians Committee for Responsible Medicine, Washington, DC, United States; [2]Green Neuroscience Laboratory, Neurolinx Research Institute, La Jolla, CA, United States
alam@pcrm.org

Induced pluripotent stem cells (iPSCs) show great potential in improving our understanding of diseases and development of therapeutics. However, a great deal of human-based iPSC research uses animal products – often without the knowledge of the researchers. We outlining how these practices have grave scientific, clinical and ethical implications. Specifically, we highlight how the field is destined to repeat failures of past models if it does not address the potential pitfalls. We then describe the collaborative development of the Xeno-Free Stem Cell (XFSC) Toolkit Initiative. This open online database is being established to help researchers and stem cell institutions: (1) identify and develop standards for XFSC protocols, (2) certify XF reagents, and (3) create an online community to increase knowledge of best practices and foster collaborations. Finally, we will describe how the field's shift to fully animal-free conditions will accelerate our progress toward autologous medicine.

VII-3-550

Toxicological assay in cultured human iPSC-derived neuronal networks using MEA system

Ikuro Suzuki, Aoi Odawara, Naoki Matsuda, Yuto Ishibashi and Remi Yokoi

Department of Electronics, Tohoku Institute of Technology, Sendai Miyagi, Japan
i-suzuki@tohtech.ac.jp

The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful *in vitro* model for evaluating drug toxicity. Epileptiform activity is one of phenomena in neuronal toxicology. We evaluated the dynamics of epileptiform activities and the effect of anti-convulsant drug in cultured hiPSC-derived neurons using high-throughput multielectrode array (MEA) system. Electrophysiological seizes were induced by Pentylentetrazole, 4-Aminopyridine, Picrotoxin, GABAgin and Kainic acid in a concentration-dependent manner. Anti-convulsant phenytoin suppressed induced epileptiform activity. However, the feature of burst firings and frequency in epileptiform activities and phenytoin administration were different with respect to each epilepsy drugs. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using high-throughput MEA system is a useful to investigate the neuronal toxicity in drug screening.

Session VII-4: 21st Century Cell Culture Practices

Co-Chairs
David Pamies, Johns Hopkins University, Baltimore, MD, United States
Anna Bal-Price, European Commission Joint Research Centre – Health, Consumers and Reference Materials, Ispra, Italy

VII-4-598

Good Cell Culture Practice (GCCP 2.0)

David Pamies[1], Anna Bal-Price[2], Sandra Coecke[2], Dave Allen[3], Glyn Stacey[4], Gerhard Gstraunthaler[5], Marcel Leist[6] and Thomas Hartung[1]

[1]EHS, CAAT, Johns Hopkins University, Baltimore, MD, United States; [2]Institute for Health and Consumer Protection Systems Toxicology Unit, Joint Research Center, ECVAM, European Commission, Ispra, Italy; [3]Integrated Laboratory Systems, Durham, NC, United States; [4]Division of Advanced Therapies, UK Stem Cell Bank, South Mimms, Hertfordshire, United Kingdom; [5]Division of Physiology, Medizinische Universität Innsbruck, Innsbruck, Austria; [6]In-vitro Toxicology and Biomedicine and CAAT-Europe, University of Konstanz, Konstanz, Germany
dpamies1@jhu.edu

In vitro cell culture technologies have been increasingly used in recent decades. The need to find cheaper, faster, humanized and more mechanistic approaches have been incentives for employing these methods in many areas, such as toxicology, drug development and disease studies. A key problem is that generally-recognized quality controls have not been established for such methods. Especially with respect to regulatory recognition, but also concerning scientific robustness, several concerns have been raised. These comprise 1) cell line authentication, 2) contaminations by microbial infection, especially with mycoplasma, 3) use of standards, references and acceptance controls to check and calibrate experimental outcomes, 4) documentation and reporting practices within laboratories and for publications. The maintenance of high standards is fundamental to all good scientific practice, and it is essential for ensuring the reproducibility, reliability, credibility, acceptance, and proper application of any results produced. In 2005, a good cell culture (GCCP) task force produced the second of two reports (Hartung et al., 2002; Coecke et al., 2005), and further updates have not taken place. Due to the fast change that *in vitro* culture has made in the last decade, new challenges in GCCP have appeared, such as stem cell-derived models and organotypic cultures. For this reason, we present here the GCCP 2.0 Collaboration. The collaboration not only plans to update the original GCCP document but also promotes its application to Toxicology for the 21st Century (Pamies et al., 2017). The aim of a renewed GCCP initiative is to reduce uncertainty in the development and application of *in vitro* procedures by encouraging the establishment of principles for the greater international harmonization, standardization, and rational implementation of laboratory practices, nomenclature, quality control systems, safety procedures, and reporting, linked, where appropriate, to the application of the principles of Good Laboratory Practice (GLP).

References

Coecke, S., Balls, M., Bowe, G. et al. (2005). Guidance on Good Cell Culture Practice – A report of the second ECVAM task force on Good Cell Culture Practice. *Altern Lab Anim 33*, 261-287.

Hartung, T., Balls, M., Bardouille, C. et al. (2002). Good Cell Culture Practice. ECVAM Good Cell Culture Practice Task Force Report 1. *Altern Lab Anim 30*, 407-414.

Pamies, D., Bal-Price, A., Simeonov, A. et al. (2017). Good Cell Culture Practice for stem cells and stem-cell-derived models. *ALTEX 34*, 95-132.

VII-4-386

Application of Good *In Vitro* Method Practices (GIVIMP) for stem-cell-derived test systems

Anna Bal-Price and Sandra Coecke

European Commission Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Ispra, Italy
anna.price@ec.europa.eu

Recently, a guidance document on Good *In Vitro* Method Practices (GIVIMP) has been developed by EURL-ECVAM and OECD to guide a use of *in vitro* methods for regulatory purposes aiming at reducing the uncertainties of produced *in vitro* data. The scope of the GIVIMP document includes the principles of good cell culture practice (GCCP) for the greater international harmonization, standardization, and rational implementation of laboratory practices. GCCP principles have to be strictly applied to pluripotent stem cell (PSC)-derived *in vitro* systems to deliver robust cellular models for toxicity testing. PSCs offer a unique opportunity to obtain various human cell types that can be exploited for human safety assessments *in vitro* and as such contribute to modern mechanistically based toxicity testing. Failure to adopt GCCP principles in laboratories significantly increases the risk of generating erroneous data as well as risking worker health issues and legal liabilities.

References

Bal-Price, A. et al. (2014). Considerations in the development of in vitro toxicity testing methods intended for regulatory use. In A. Bal-Price and P. Jennings (eds.), *In Vitro Toxicology Systems, Methods in Pharmacology and Toxicology*. New York: Springer.

Coecke, S., Balls, M., Bowe, G. et al. (2005). Guidance on Good Cell Culture Practice – A report of the second ECVAM task force on Good Cell Culture Practice. *Altern Lab Anim 33*, 261-287.

Pamies, D., Bal-Price, A., Simeonov, A. et al. (2017). Good Cell Culture Practice for stem cells and stem-cell-derived models. *ALTEX 34*, 95-132.

Suter-Dick, L. et al. (2015). Stem cell-derived systems in toxicology assessment. *Stem Cells Dev 24*, 1284-1296.

VII-4-523

Development of Good Cell Culture Practice for new *in vitro* techniques: The GCCP 2.0 Collaboration

Glyn Stacey[1], Anna Bal-Price[2], Sandra Coecke[2], Gerhard Gstraunthaler[3], Thomas Hartung[4], Marcel Leist[5], David Allen[6] and David Pamies[4]

[1]UK Stem Cell Bank, NIBSC, South Mimms, Herts, United Kingdom; [2]Institute for Health and Consumer Protection Systems Toxicology Unit, Joint Research Center, ECVAM, Ispra, Italy; [3]Department of Physiology and Medical Physics, Medical University of Innsbruck, Innsbruck, Austria; [4]Center for Alternatives to Animal Testing, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States; [5]In-vitro Toxicology and Biomedicine and CAAT-Europe, University of Konstanz, Konstanz, Germany; [6]Integrated Laboratory Systems Inc., Research Triangle Park, NC, United States
glyn.stacey@nibsc.org

Technologies for *in vitro* cell culture technologies have been dramatically developed in recent decades. The need to find cheaper, faster, humanized and more mechanistic approaches have been incentives for employing these methods in many areas such as toxicology, drug development and disease studies. A key problem is that there is all too often a lack of attention to fundamental quality control when using these methods. There are a number of key concerns which have raised the awareness of challenges for scientific quality in cell culture experiments. Some of the major concerns include: 1) lack of cell line authentication, 2) contamination by microorganisms which are commonplace and have a serious impact on *in vitro* results, especially with mycoplasma, 3) documentation and reporting practices in laboratory work and publications, which are often of limited quality.

In 2005, a GCCP task force produced the last of two reports (Hartung et al., 2002; Coecke et al., 2005). The maintenance of high standards is fundamental to all good scientific practice, and it is essential for assuring reproducibility, reliability, credibility, acceptance, and proper application of any results produced. The aim of GCCP is to reduce uncertainty in the development and application of *in vitro* procedures by encouraging the establishment of principles for the greater international harmonization, standardization, and rational implementation of laboratory practices, nomenclature, quality control systems, safety procedures, and reporting, linked, where appropriate, to the application of the principles of Good Laboratory Practice (GLP).

The significant developments for *in vitro* culture in the last decade present new challenges in GCCP, such as stem cell-derived models and organotypic cultures. For this reason, we present here the GCCP 2.0 COLLABORATON (Pamies et al., 2017) which not only plans to update the original GCCP document but also to promote its application to Toxicology for the 21st Century.

In particular the presentation will cover the following aspects:

1. GCCP as a crucial underpinning activity and way of thinking in research as well as formal *in vitro* testing and manufacture under formal Quality Assurance
2. Its core components and exemplars of consequences of failure to implement GCCP
3. Application of GCCP in research, testing and manufacture of cell derived products and cell-based medicines
4. New challenges in use of stem cell and 3D culture and analytics based on omics technology.

References

Coecke, S., Balls, M., Bowe, G. et al. (2005). Guidance on Good Cell Culture Practice. A report of the second ECVAM Task Force on Good Cell Culture Practice. *Altern Lab Anim 33*, 1-27.

Pamies, D., Bal-Price, A., Simeonov, A. et al. (2017). Good Cell Culture Practice for stem cells and stem-cell-derived models. *ALTEX 34*, 95-132.

VII-4-397

Systematic characterization of microphysiological systems (MPS)

Murat Cirit
MIT, Cambridge, MA, United States
mcirit@mit.edu

A large percentage of drug candidates fail at the clinical trial stage due to a lack of efficacy and unacceptable toxicity, primarily because the *in vitro* cell culture models and *in vivo* animal models commonly used in preclinical studies provide limited information about how a drug will affect human physiology. The need for more physiologically relevant *in vitro* systems for preclinical efficacy and toxicity testing has led to a major effort to develop "Microphysiological Systems (MPS)" based on engineered human tissue constructs. The MPS development process requires an initial assessment of viability and functionality, followed by an examination of the MPS response to various stimuli, including drugs, toxins, and disease-related cues. These extensive development efforts take place mainly in the developer's lab, and the reproducibility of the MPS results are rarely assessed by an independent research group or transferred to industry partners for use in drug development. Although there is a need for more physiologically-relevant preclinical testing technologies, the transition of MPS technologies from academia to industry remains challenging. Successful transfer and deployment of MPS technologies requires quantitative characterization and validation of the systems, preferably by an independent and unbiased external testing facility. The Translational Center of Tissue Chip Technologies is to fill this gap between academic research and development and industrial application of MPS technologies The Translational Center for Tissue Chip Technologies combines a holistic and mechanistic approach – based on quantitative systems pharmacology (QSP) – that combines quantitative experimental biology, computational biology, and biostatistics to achieve unbiased characterization of these complex systems and translation of experimental insights to clinical outcomes.

VII-4-772

Evaluation of CNS synaptic dysfunction using drebrin immunoreactivity

Yuko Sekino[1] and Tomoaki Shirao[2]

[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan; [2]Dept Neurobiology & Behavior, Gunma University Graduate School of Medicine, Maebashi, Japan

yukos@mol.f.u-tokyo.ac.jp

Prediction of adverse effects of chemicals on the central nervous system with experimental animals has the limitation on higher brain function, such as learning and memory. Thus, mechanism-based *in vitro* assays are strongly needed. Drebrin A is a neuron-specific actin binding protein located in excitatory postsynaptic structures of matured neurons. The loss pf drebrin clusters is known as a good marker of synaptic dysfunctions. Frozen hippocampal neurons prepared from E18 rat brains (SKY-neurons) were cultured for 21 days and drebrin clusters were counted immunohistochemically to reveal the toxicity of Amyloid beta (Aβ). The number of the clusters decreases by the soluble oligomers (Aβ-derived diffusible ligands, ADDLs). The acute decrease of drebrin clusters was not due to the cell death. Further, the toxicity of ADDLs is inhibited by histone deacetylase inhibitor, suberoylanilide hydroxamic acid. In this talk, the application of drebrin immunohistchemistly to the human iPS-derived neurons will be discussed.

References

Ishizuka, Y., Shimizu, H., Takagi, E. et al. (2014). Histone deacetylase mediates the decrease in drebrin cluster density induced by amyloid beta oligomers. *Neurochem Int 76*, 114-121.

Ohara, Y., Koganezawa, N. and Yamazaki, H. (2015). Early-stage development of human-induced pluripotent stem cell (hiPSC)-derived neurons. *J Neurosci Res 93*, 1804-1813.

VII-4-245

Towards the truly non-animal lab – The issue with animal derived reagents in cell culture

Roman Kolar and Carolin Spicher

Animal Welfare Academy, German Animal Welfare Federation, Neubiberg, Germany

roman.kolar@tierschutzakademie.de

Animal derived reagents like sera, antibodies, enzymes and cell attachment factors – some even sourced from live animals – are routinely used in biomedical labs, particularly those working *in vitro*. From an animal welfare perspective, subjecting animals to harm to obtain reagents which can be substituted with non-animal derived alternatives is ethically unacceptable (Jochems et al., 2002). In addition there are various scientific problems, for example a lack of reproducibility or the misinterpretation of results, which can be caused by batch to batch variations of reagents or the application of chemically undefined media in cell culture (van der Valk et al., 2004). On that account, we describe available alternatives and their advantages and/or possible limitations in common cell culture. The need to overcome the lack of regulations in this context will also be addressed. Our aim should be to remove redundant animal-derived reagents from labs to ensure high quality science that doesn't interfere with animal welfare.

References

Jochems, C. E., van der Valk, J. B., Stafleu, F. R. and Baumans, V. (2002). The use of fetal bovine serum: Ethical or scientific problem? *Altern Lab Anim 30*, 219-227.

van der Valk, J., Mellor, D., Brands, R. et al. (2004). The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. *Toxicol In Vitro 18*, 1-12.

VII-4-250

Adaptation of the human Cell Line Activation Test (h-CLAT) to animal product-free conditions

Alexander Edwards, Lottie Roscoe, Christopher Longmore, Bushra Sim and Carol Treasure

XCellR8, Daresbury, United Kingdom

alex.edwards@x-cellr8.com

THP-1 cells were adapted to animal free (AF) culture using 10% Human Serum instead of FCS. BSA in the washing and staining buffer was replaced with Human Serum Albumin and custom anti-CD54 and 86 antibodies from a non-animal source were used. Adaptations to the h-CLAT were assessed using the proficiency chemicals from OECD TG442E. THP-1 cells in AF culture exhibited comparable doubling times (mean: 44 hrs) and morphology. Mean cell numbers after 48 hr and 72 hr pre-culture with initial seeding density at 0.2 x 10^6 cells/ml were 0.41 and 0.61 x 10^6 cells/ml, respectively. CV75 values (dose yielding 75% viability) and changes in CD54 and 86 expression were assessed in the animal free system using the proficiency chemicals from TG442E. The AF version of the h-CLAT met the criteria for demonstrating proficiency and was successfully validated for use. These modifications have received approval for use in REACH submissions and we are currently seeking approval for inclusion in OECD TG442E.

Session VII-5: Implications of Systematic Review

Co-Chairs
Andrew Kraft, U.S. Environmental Protection Agency, Washington, DC, United States
Marlies Leenaars, SYRCLE Radboudumc, Nijmegen, The Netherlands

VII-5-757

The rise of systematic review methods in environmental health sciences

Andrew Kraft
U.S. Environmental Protection Agency, Washington, DC, United States
kraft.andrew@epa.gov

There is an acknowledged need to apply tenets of systematic review initially developed for use in evidence-based medicine to the conduct and analysis of environmental health studies. These studies of chemical exposure are more heterogeneous than clinical trials and the data available vary across a wide range of study types (e.g., *in silico*; high-throughput *in vitro*; animal bioassay; epidemiology). This talk will highlight the ongoing emergence of international collaborations and tools focused on applying systematic review methods to animal research. By extension, it will discuss how applying these methods to the review of studies of environmental chemicals might inform the development of alternatives to reduce, refine, and replace the use of animals in research. An emphasis will be placed on the difficulties inherent to applying systematic review methods to the review of environmental health studies, as well as potential solutions.

Disclaimer: The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

VII-5-417

Implications of systematic review for the conduct, reporting, and evaluation of studies

Marlies Leenaars
SYRCLE Radboudumc, Nijmegen, The Netherlands
marlies.leenaars@radboudumc.nl

Methodology and reporting of animal studies is currently inadequate and improvements are urgently needed (Lancet Series, 2014). Systematic reviews can help improve this situation by making current practice in animal research transparent and thereby raising awareness. In 2011 the Montréal Declaration on the synthesis of evidence to advance the 3Rs principles in science was adopted at the 8th World congress on Alternatives (Leenaars et al., 2012). Besides stimulating better science, the potential benefits of systematic reviews encompass: (1) leading to better informed ethical review, (2) helping to implement the Three Rs, and (3) improving translational transparency to inform clinical trials (Ritskes-Hoitinga et al., 2014). Education and training is already available and needs further development and widespread distribution worldwide to make systematic reviews common practice. Methodology to conduct systematic reviews and to assess translational value of animal studies are being developed.

References

Lancet Series (2014). Research: Increasing value, reducing waste. *Lancet 383*, 156-185.

Leenaars, M., Ritskes-Hoitinga, M., Griffin, G. and Ormandy, E. (2012). Background to the Montréal Declaration on the Synthesis of Evidence to advance the 3R principles in science, as adopted by the 8th World Congress on Alternatives and Animal Use in the Life Sciences, Montréal, Canada, August 25, 2011. *ALTEX Proc 1*, 35-38.

Ritskes-Hoitinga, M., Leenaars, M., Avey, M. T. et al. (2014). Systematic reviews of preclinical studies can make significant contributions to health care and more transparent translational medicine (editorial). *Cochrane Database Syst Rev 3*, ED000078.

VII-5-766

Toward a better translation of animal experimental data into clinical use: Outlines of translational strategies

Frans Stafleu[1], Franck Meijboom[1], Merel Ritskes-Hoitinga[2], Cathalijne Leenaars[2] and Tineke Coenen de Roo[3]
[1]Ethics Institute, Utrecht University, Utrecht, The Netherlands; [2]SYRCLE Radboudumc, Nijmegen, The Netherlands; [3]Coenen Consultency, Maren-Kessel, The Netherlands
f.r.stafleu@uu.nl

A growing amount of literature shows that the translation of animal experimental data into clinical use is low. In our research project "from animal model to translational strategy" we try to develop "translational strategies" that include a range of measures covering the total research chain and aim to help improving the success rates concerning the translation of (animal) experimental data into the clinic.

One of the reasons for the low translational value starts in lack of recognition that an experimental animal is not a human patient. The differences between the standardised experimental animal (e.g., one species, one inbred strain, one gender, young, healthy) and the patient (outbred, old/young, male and female, comorbidity etc.) are huge. However, based on scientific arguments and the ethical imperative to reduce the number of animals there still is a demand for standardised animals. In this way standardisation of experimental animals seems to counteract the chances for a successful translation

to human patients. We have called this the standardisation-translation paradox (Stafleu et al., 2016).

How can we tackle this paradox? The challenge is to incorporate the complexity of the patient into the research. Although our project is not yet finished a few possibilities to do this are emerging.

Reversed translation: Reversed translation means that the research question is formulated in the clinic and than translated into a question that can be addressed with the help of an animal experiment.

Starting in the clinic, means starting with the patient and therefore patient participation is of enormous value in this phase of research development. One of the mistakes which patient participation may help to avoid is that a research question is formulated which is not relevant for the patient and/or the clinic. Reversed translation has become more feasible through the increased availability of technics which collect all kinds of data of the patient and her disease. Examples are genetic information, blood parameters like enzymes and results of different kinds of images techniques. These data can be incorporated in "big data" and subsequently be analysed and translated into specific research questions apt for animal research.

Standardised variation: To mimic the patient in animal research we have to incorporate in a standardized manner the variation we find in the patient into our research. The challenge is to find the relevant characteristics to variate with. In the process of the reversed translation a list must be made of relevant variations (e.g., gender, genetic background, humidity) which has to be incorporated into the animal research. This must be done in a standardized way meaning that the variation is within the groups not between the groups.

Good practice: Although not part of "incorporating the complexity of the patient into the research", good methodology while performing the animal experiment is certainly part of translational strategies. Many experiments are still performed without proper blinding, randomisation, statistical methods etc. This results in not validated data which will only by change relevant for clinical use.

Integration of the research chain: Integration of the research chain is for two reasons important. The first reason is the need for reversed translation. To enable this "vertical" integration of preclinical and clinical research is necessary. Doctors and patients have to work in close collaboration to collect the clinical data and to translate them in preclinical research questions. But also in the preclinical phase the integration of research is necessary. Modern translational research makes use of many different disciplines like animal experimenting, organs on a chip, omics etc. We call this "horizontal integration". This means that meaningful research has to be done in consortia which are horizontally and vertically integrated.

The development of translational strategies is funded by the Netherlands Organisation for Scientific Research (NWO_313-99-310)

VII-5-34

Application of systematic reviews for transparent, objective and consistent test methods comparison to inform regulatory decisions about new test methods acceptance

Sebastian Hoffmann[1], Katya Tsaioun[2] and Martin Stephens[2]

[1]seh consulting + services, Paderborn, Germany; [2]EBTC at Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

sebastian.hoffmann@seh-cs.com

Alternative approaches to the use of vertebrate animals in toxicology are needed for environmental hazard and risk assessments of chemicals under the ethical, legal, financial and public pressure. While our understanding of toxicity mechanisms has advanced, less progress has been made in acceptance and standardization of new tests, pointing to a disconnect between the advancements in the toxicological and regulatory sciences. Evidence-Based Methodologies (EBM) and their principal tool, a systematic review (SR), can bridge this gap. The principles of EBM will be described and an example of the application of this approach as a SR of zebrafish embryotoxicity test method will be presented. In this SR a multi-stakeholder working group has developed a protocol for comparison of zebrafish embryotoxicity test and the standard mammalian test TG-414. The results of the pilot test and challenges of pioneering this application of SR in toxicology will be presented. Best practices of SR in toxicology will be proposed.

References

Rooney, A. A., Boyles, A. L., Wolfe, M. S. et al. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. *Environ Health Perspect 122*, 711-718.

Samuel, G. O. et al. (2016). Guidance on assessing the methodological and reporting quality of toxicologically relevant studies: A scoping review. *Environ Int 92-93*, 630-646.

Stephens, M. L. et al. (2016). The emergence of systematic review in toxicology. *Toxicol Sci 152*, 10-16.

VII-5-678

Pros and cons of systematic review

Emily Sena

University of Edinburgh, Edinburgh, United Kingdom

sena.emily@gmail.com

Systematic reviews have proven useful in quantifying the presence and impact of potential sources of bias in animal research. Specifically, we have identified that only a third of studies report randomisation and blinding, and less than 1% report a sample size calculation. Studies at high risk of bias are associated with overestimating effect sizes. In an assessment of publication bias in the *in vivo* stroke literature we estimate that 1 in 6 experiments remains unpublished and this leads overestimation of around 30% in reported treatment effects. This work has led to evidence-based guidelines for the conduct and reporting of animal studies to improve their validity. However, there are a number of limitations of systematic review such as the rate at which they can become outdated, only being as good as the primary data included and their mass production that may limit their utility and impact. Recent methodological advances such as machine learning, text mining and online repositories have recently been applied to address these limitations and ensure their continued relevance. As a field it in important to ensure they are warranted, of high quality and fulfil a purpose.

Session VII-6: IVIVE Approaches

Co-Chairs
Nisha Sipes, NTP/NIEHS, Research Triangle Park, NC, United States
Nynke Kramer, Utrecht University, Utrecht, The Netherlands

VII-6-151

Challenges and opportunities in using IVIVE to quantify risk and improve decision-making

Weihsueh Chiu
Texas A&M University, College Station, TX, United States
wchiu@cvm.tamu.edu

Like traditional toxicity testing methods, alternative methods have often been utilized to identify hazards to human health. The recent successes related to screening of estrogenic compounds using a suite of *in vitro* assays have also been based on successful discrimination between known "active" and "inactive" compounds. However, many decisions related to chemical exposures are based on risk, rather than hazard alone, and progressing from hazard to risk necessitates going beyond "activity" calls to quantitative estimates of potency. Generally accepted practices exist for extrapolating traditional toxicity testing data to estimate human risk, but not for *in vitro*-to-*in vivo* extrapolation (IVIVE). This presentation reviews several key challenges to application of IVIVE to quantify risk, such as to addressing variability in susceptibility and prediction uncertainty, as well as approaches to address these challenges, such as population-based data and analyses and probabilistic methods.

VII-6-130

IVIVE methods to predict human interaction likelihood with the Tox21 10k library

Nisha Sipes
NTP/NIEHS, Research Triangle Park, NC, United States
nisha.sipes@nih.gov

In vitro-in vivo extrapolation (IVIVE) analyses are providing hazard-based context to *in vitro* assay data, which is specifically useful when *in vivo* data are not available. Exposure models, like the US EPA's ExpoCast program, are predicting median daily exposures for thousands of chemicals. Comparing the two provides a fit-for-purpose first glance at the potential risk associated for the median human population. We present a data driven approach to relate *in vitro* responses (AC_{50}s & efficacies) to human *in vivo* interactions using *in silico* parameters across the entire Tox21 federal collaboration 10k chemical library, toward a more rapid human hazard assessment. This approach is similar to previous endeavors, but takes into consideration *in vitro* response efficacies, concentration-response fit quality, likelihood of *in vivo* interactions based on a clinical context, and extends the approach for the first time to the entire Tox21 high-throughput screening dataset.

This abstract does not reflect the views of the NTP or US EPA.

VII-6-203

An open-source IVIVE workflow integrating QSAR and PK models

Xiaoqing Chang[1], Qingda Zang[1], Shannon Bell[1], Dave Allen[1], Warren Casey[2] and Nicole Kleinstreuer[2]
[1]ILS, Morrisville, NC, United States; [2]NIH/NIEHS/DNTP/NICEATM, RTP, NC, United States
changx@niehs.nih.gov

Many chemicals in commerce lack safety information. Accurate estimates of *in vivo* toxicity for these chemicals are needed to inform decisions on safe handling and use as well as accidental exposure responses. To address this need, we have developed an open-source *in vitro* to *in vivo* extrapolation (IVIVE) workflow incorporating pharmacokinetic (PK) models with differing complexities. The IVIVE workflow allows prediction of external dose corresponding to a predefined plasma concentration derived from *in vitro* assay data, or estimation of plasma concentration following a given dose. We developed a set of QSAR models and embedded them in the workflow to provide PK model input parameters such as fraction unbound to plasma proteins, partition coefficients, and Henry's constant. Evaluation of these models' performance yields R^2 values of 0.742-0.861 compared to experimental measurements.

This project was funded with U.S. Federal funds from NIEHS/NIH/HHS under Contract HHSN273201500010C.

VII-6-459

IVIVE and PBPK modelling to support *in vitro*-based chemical safety assessment

Miyoung Yoon[1], Gina Song[1], Marjory Moreau[1], Pankajini Mallick[1], Alina Efremenko[1], Jerry Campbell[2] and Harvey Clewell[1]

[1]Biokinetics and Biosimulations, ScitoVation, LLC, Research Triangle Park, NC, United States; [2]Ramboll Environ, Research Triangle Park, NC, United States

myoon@scitovation.com

To use *in vitro* data in chemical safety assessment, *in vitro* data on bioactivity concentrations need to be translated into human *in vivo* exposures. PBPK models provide an effective tool for conducting such IVIVE when used within AOP framework. Their physiological structure facilitates the incorporation of *in silico*- and *in vitro*-derived chemical-specific parameters in order to predict *in vivo* absorption, distribution, metabolism and excretion of the chemicals. PBPK models can be used to predict *in vivo* exposure conditions that would produce chemical concentrations in the target tissue equivalent to the concentrations at which effects were observed in *in vitro* toxicity assays. They can also support the identification of potentially susceptible populations associated with age-dependent pharmacokinetics or metabolic polymorphisms. This presentation will discuss the current issues in IVIVE and the research efforts to address those to move forward. Two examples of IVIVE-PBPK modeling will be presented, one for parabens margin of safety analysis and the other for pesticides early life sensitivity evaluation.

VII-6-587

Evaluating high throughput toxicokinetics and toxicodynamics for IVIVE

John Wambaugh[1], Robert Pearce[1], Gregory Honda[1], Michael Hughes[2], Caroline Ring[1], Lyly Pham[1], Barbara Wetmore[3] and Woodrow Setzer[1]

[1]National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC, United States; [2]National Health and Environmental Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC, United States; [3]National Exposure Research Laboratory, U.S. EPA, Research Triangle Park, NC, United States

wambaugh.john@epa.gov

High-throughput screening (HTS) generates *in vitro* data for characterizing potential chemical hazard. TK models are needed to allow *in vitro* to *in vivo* extrapolation (IVIVE) to real world situations. The U.S. EPA has created a public tool (R package "httk" for high throughput toxicokinetics) for TK and physiologically-based TK (PBTK). We are now able to rapidly parameterize generic PBPK models using *in vitro* data to allow IVIVE for 543 chemicals. We evaluate using four R's: We have (1) Reused existing TK data by compiling a library of TK time course data in, this data has (2) Refined the design of *in vivo* TK studies, allowing us to perform new, informative experiments for high value chemicals using a (3) Reduced (n = 6) study design. Careful evaluation of the existing and new data allows comparison of the results of *in vitro* HTS bioactivity assays with previously collected *in vivo* toxicity studies. In some cases, we may be able to (4) Replace *in vivo* animal studies with HTS and HTTK.

This abstract does not necessarily reflect U.S. EPA policy.

VII-6-511

The influence of *in vitro* kinetics on cytotoxic potency rankings

Nynke Kramer, Floris Groothuis and Bas Blaauboer

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

N.I.Kramer@uu.nl

Despite similar nominal effect concentrations, the biologically effective concentration in cells may vary significantly between chemicals and between *in vitro* and *in vivo* assay setups. Not considering differences in biologically effective concentrations between assays hampers quantitative *in vitro-in vivo* extrapolations (QIVIVE). The influence of the *in vitro* distribution on *in vitro* readout has been studied for neutral organic chemicals, but not for complex chemicals like surfactants. Here we present the results from our study where we measure and compare the extent to which *in vitro* setup influences the distribution and readout of neutral chemicals and charged surfactants in an RTgill-W1 cytotoxicity assay. Results indicate that cytotoxic potency rankings vary significantly depending on the dose-metric used to make the ranking and that a chemical's membrane-water partition coefficient may be used to estimate *in vitro* biologically effective concentrations.

Session VII: Translation

Poster Presentations

VII-29

Study of *in vitro* irritation testing in feminine hygiene products using a reconstructed human vaginal epithelium model

Hidefumi Ikeda, Shoko Koizumi and Masayuki Takaishi
Product Assurance Division, Mandom Corp., Osaka, Japan
ikeda-hi@mandom.co.jp

There is an increasing need to evaluate feminine hygiene products for vaginal, in addition to skin, irritation. In the past, testing of such products was performed using an *in vivo* vaginal irritation test. In a recent study, a reconstructed human vaginal epithelium model was developed as an *in vitro* evaluation method (Fichorova et al., 2004; Ayehunie et al., 2011). However, few reports validating this method have been published. In this study, we compared the data for the new vaginal irritation test (SkinEthic HVE) with those for the *in vivo* rabbit irritation test and the previously reported vaginal irritation test. We tested bactericidal materials, surfactants, and commercial feminine hygiene products. We evaluated vaginal irritation by measuring ET50 using the WST-8 assay. Data for the new vaginal irritation test were similar to those for the previously reported vaginal irritation test. Our results suggest that irritation testing using the SkinEthic HVE model can provide useful risk assessment for feminine hygiene products and their ingredients.

References

Ayehunie, S., Cannon, C., Larosa, K. et al. (2011). Development of an in vitro alternative assay method for vaginal irritation. *Toxicology 279*, 130-138.

Fichorova, R. N., Bajpai, M., Chandra, N. et al. (2004). Interleukin (IL)-1, IL-6, and IL-8 predict mucosal toxicity of vaginal microbicidal contraceptives. *Biol Reprod 71*, 761-769.

VII-32

Hypothermia protects retinal cells against cobalt-chloride induced hypoxic damage in a porcine organ explant culture

Stephanie Joachim[1], Sandra Kuehn[1], Ana Maliha[1], Jose Hurst[2], Teresa Tsai[1] and Sven Schnichels[2]
[1]Experimental Eye Research, Ruhr-University Bochum, Bochum, Germany; [2]University Eye Hospital Tübingen, University of Tübingen, Tübingen, Germany
stephanie.joachim@rub.de

Purpose: Cobalt-chloride ($CoCl_2$) induces a strong degeneration of the porcine retina through hypoxic mechanisms. Neurons of the inner retina layers are particularly affected. This model is used to study possible protective effects of hypothermia treatment.

Methods: Organotypic cultures of porcine retinas were cultured and treated with 300 µM $CoCl_2$ for 48 h. Hypothermia (30°C) was applied for the same duration.

Results: At day 8, RGC layer thickness decreased. In accordance, a loss of retinal ganglion cells (RGCs) was noted in the $CoCl_2$ group ($p = 0.0004$), while RGCs were rescued through hypothermia. The amount of apoptotic RGCs was higher in the $CoCl_2$ group ($p = 0.0002$), but also present in the $CoCl_2$+hypothermia group ($p = 0.002$).

Conclusions: A protection of RGCs could be achieved through hypothermia application in a hypoxia porcine retina organ culture. This alternative model is suitable for drug and treatment screening and will therefore reduce the number of animal studies.

VII-42

Surplus animal and tissue share program at the University of Saskatchewan (UofS)

Amanda Plante and Jane Alcorn
University of Saskatchewan, Saskatoon, SK, Canada
uacc.office@usask.ca

Russell and Burch's 3Rs tenet (Replacement, Reduction and Refinement) guide the ethical use of animals in science. To promote the 3Rs, the UofS University Animal Care Committee (UACC) developed a formal program in 2013 to implement a reuse and reduction in animal numbers. This program involves a Surplus Animal/Tissue Share Animal Use Protocol (AUP) and a centralized request and donation system. Use of surplus live animals or bodily fluids/tissues from euthanized animals (negative control experimental groups or unused animals from breeding/production colonies) associated with UACC approved AUPs is permitted under this Animal/Tissue Share AUP. Requests for and donations of animals/tissues are submitted via an online form and are managed and tracked by UACC Animal Technicians in consultation with Facility Managers and the University Veterinarian. The UofS Animal/Tissue Share Program serves as a best practice model that can be incorporated into other institution's animal use programs.

VII-47

The study of technical transferability and inter-laboratory reproducibility of the SH test (Part 1)

Kaoru Kasahara[1], Maki Sugiyama[1], Akemi Toyoda[1], Noriyasu Imai[2], Sakiko Aizawa[2], Midori Takeyoshi[2] and Morihiko Hirota[3]
[1]POLA Chemical Industries, Inc., Yokohama, Japan; [2]KOSÉ Corporation, Tokyo, Japan; [3]Shiseido Global Innovation Center, Yokohama, Japan
k-kasahara@pola.co.jp

Development of alternative methods is an urgent issue for the safety assessment of cosmetic products and ingredients. The SH test (Hirota et al., 2013) is one of the alternative methods for skin sensitization, detecting the changes of cell-surface thiols by the treatment with haptens. We have evaluated the SH test from the viewpoint of technical transferability since 2015 (Takeyoshi et al., 2015) and revealed some factors that affected the results.

The SH test was performed according to the published reports (Hirota et al., 2013). Firstly, we evaluated DNCB (2,4-dinitrochlorobenzene), which is a positive control, and compared the changes of cell-surface thiols among 3 laboratories. Secondly, the test conditions were refined using other test substances which did not show inter-laboratory reproducibility.

All 3 laboratories obtained the same results in dose-response changes of cell-surface thiols induced by DNCB, which indicated equivalent operability. Several test substances were predicted correctly by making pH neutral or changing the maximal applied concentration. The results suggest that the refined test conditions can improve technical transferability.

References

Hirota, M., Kouzuki, H., Ashikaga, T. et al. (2013). Artificial neural network analysis of data from multiple in vitro assays for prediction of skin sensitization potency of chemicals. *Toxicol In Vitro 27*, 1233-1246.

Takeyoshi, M. et al. (2015). JSAAE 28th Annual Meeting.

VII-60

Evaluation of skin sensitizer potency prediction model combining multiple *in vitro* tests

Akemi Toyoda, Kaoru Kasahara, Maki Sugiyama and Koichi Nomura
POLA Chemical industries, Inc., Yokohama, Japan
a-toyoda@pola.co.jp

Recently there have been multiple alternatives to skin sensitization tests guidelines are progressing. Furthermore, investigations into combined sensitivity potency evaluation of these test methods have started to be reported. In this study, we constructed a new formula for predicting the LLNA EC3 value from multiple *in vitro* skin sensitization test results, and evaluated the predictability.

We constructed a prediction formula from a multiple regression analysis using a data set from the results of SHtest, KeratinoSens™ , and h-CLAT for 136 chemicals. This regression equation sets LLNA EC3 as the objective variable and each measured value of SHtest, KeratinoSens™ , and h-CLAT as explanatory variables, and statistical analysis software JMP (ver.11.0.0) was used for analysis.

The concordance rate with the LLNA potency categories was 66.2%, with 26.5% Over predict and 7.4% Under predict. There was a correlation between the actual measurement and predicted measurement with the prediction formula constructed based on the data set used here and we confirmed that it generally had a good level of predictability.

References

Hirota, M., Fukui, S., Okamoto, K. et al. (2015). Evaluation of combinations of in vitro sensitization test descriptors for the artificial neural network-based risk assessment model of skin sensitization. *J Appl Toxicol 35*, 1333-1347.

Urbisch, D., Mehling, A., Guth, K. et al. (2015). Assessing skin sensitization hazard in mice and men using non-animal test methods. *Regul Toxicol Pharmacol 72*, 337-351.

VII-85

Development of a targeted mass spectrometry protein assay to identify early stages of pulmonary response to carbon nanotube exposure in a 3D lung model

Gina Hilton[1], Hana Barosova[2], James Bonner[1], Barbara Rothen-Rutishauser[2] and Michael Bereman[1]

[1]Biological Sciences, North Carolina State University, Raleigh, NC, United States; [2]Université de Fribourg, Adolphe Merkle Institute, Fribourg, Switzerland

gmhilton@ncsu.edu

Introduction: Targeted mass spectrometry (MS) based proteomics is being increasingly investigated for its notable applications in rigorous assay development. One of the greatest advantages of proteomics is its ability to provide specific, reproducible, and quantitative measurements for most proteins of interest. MS technologies are rapidly developing towards sensitive detection ranges that are competitive with traditional antibody immunoassays, and thus have been implicated in clinical studies for the replacement of immunoassays for biomarker development. This study aims to use the guidelines created in the clinical community to generate a robust targeted MS assay to be used for toxicity screening of various nanomaterials.

Method: 3D lung model assembled of human-originating cell lines was exposed to Multi-walled carbon nanotubes (MWCNTs) under submerged conditions at both a 24 hr and 96 hr time-points. Cells and media were collected for each exposure. Both global and targeted methods were used to assess proteome changes for each sample. An internal standard spike-in was used to normalize all proteomic results.

Results: An average of 2795 proteins were found in cell lysate, and 755 proteins found in serum-free media using a global MS method. Significant changes were found in the exposure group at both 24 hr and 96 hr in pathways related to lung injury. Media samples were additionally tested in a targeted MS method to monitor 25 proteins of interest related to inflammatory and fibrotic response; including: TGF-β, IL-1β, IL-6, and several collagen proteins. Differential secreted protein expression was found to be increased in the exposure group compared to control, and increased in 96 hr compared to 24 hr.

Conclusion: The 3D lung model exposed to MWCNTs proved to be a successful model for the assessment of cellular mechanistic response to pulmonary injury; as well as toxicity testing for assay development. Further testing is currently being conducted using an air-liquid interface exposure. Additional experimentation will be used to find proteins that meet criteria set by the clinical MS community to be used as a robust targeted toxicity testing assay.

Supported by NIEHS grants T32 ES007046 and the Peta International Science Consortium.

VII-105

Increasing use of human tissue to improve translation in biological and pharmaceutical research

Sam Jackson, Helen Prior, Anthony Holmes and Vicky Robinson

National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, United Kingdom

sam.jackson@nc3rs.org.uk

Human tissue use has expanded rapidly across a range of research areas, but a coordinated approach to application in human disease and safety modelling is lacking. The more widespread development and use of human tissue models would allow replacement of some less translationally-relevant animal models. The NC3Rs has embarked on a series of projects to identify the hurdles researchers face when using a human tissue-based model and how these might be overcome to increase uptake of these approaches. Issues consistently raised across these programmes as being key barriers to wider adoption include access to tissue, logistics, incomplete patient data and experimental practicalities.

In response, the NC3Rs is connecting organisations and researchers working with human tissue to overcome the barriers and promote data sharing initiatives which will be described here. For example, the NC3Rs Human Tissue Hub provides information and case studies on the application of human tissue in research. An increase in the uptake of human tissue-based models could improve the human relevance of preclinical modelling, and reduce the number of animals used in preclinical testing.

References

Edwards, J., Belvisi, M., Dahlen, S. E. et al. (2015). Human tissue models for a human disease: What are the barriers? *Thorax 70*, 695-697. doi:10.1136/thoraxjnl-2014-206648

Holmes, A., Bonner, F. and Jones, D. (2015). Assessing drug safety in human tissues – What are the barriers? *Nat Rev Drug Discov 14*, 585-587. doi:10.1038/nrd4662

VII-109

The use of Göttingen Minipigs as an alternative species in scientific research

Lars Friis Mikkelsen

Ellegaard Göttingen Minipigs, Dalmose, Denmark
lfm@minipigs.dk

In the 1960s, the Göttingen Minipig was developed at the University of Göttingen in Germany to meet the demand for a non-rodent animal model with many similarities to humans. In the early 1990s, the global right to breed and sell barrier-bred microbiologically defined Göttingen Minipigs was acquired by Ellegaard Göttingen Minipigs in Denmark. Minipigs are an important animal model for biomedical research. In recent years, Göttingen Minipigs have emerged as an alternative to dogs and non-human primates in both preclinical toxicology and safety testing plus as an animal model for pharmacological research. Today, the Göttingen Minipigs are the most well characterized and most commonly used minipig for biomedical research within a number of disease and therapeutic areas like diabetes and obesity, cardiovascular diseases, gastrointestinal diseases, skin diseases, eye conditions, CNS and anticancer treatment. The translational value of the Göttingen Minipig is mainly due to its similar anatomical and physiological characteristics and important genetic similarities, which all fulfil the industry's wish for an animal model with a high translational value.

Reference

Webster, J., Bollen, P., Grimm, H. et al. (2010). Ethical implications of using the minipig in regulatory toxicology studies. *J Pharmacol Toxicol Methods 62*, 160-166. doi:10.1016/j.vascn.2010.05.002

VII-139

Comparing IVIVE outcomes of PFOA-induced nephrotoxicity using three kidney cell lines

Femke Taverne, Nynke Kramer and Bas Blaauboer

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
f.j.taverne@uu.nl

The future of toxicological risk assessment is shifting towards non-animal test approaches including *in vitro* cell based assays and quantitative *in vitro-in vivo* extrapolation (QIVIVE) models. The cell type used in *in vitro* assays may substantially influence the predicted animal-equivalent dose due to, for example, variations in tissue specific expression of xenobiotic transporters. Here we present results of a QIVIVE study for perfluorooctanoic acid (PFOA) in rats. Nominal, free and cell-associated cytotoxic concentrations of PFOA were measured and compared in HEK, NRK-52E and RPTEC/TERT1 cells and used to predict equivalent oral toxic doses in rats by means of a physiologically based pharmacokinetic model (PBPK). When *in vivo* organic anion transporter (OAT) affinity and expression levels are known, *in vitro* cell-associated concentrations best predicted *in vivo* toxic doses. When affinity for OAT transporters is unknown, RPTEC/TERT1 was the most suitable model for IVIVE of PFOA.

VII-153

High-content screening identifies two nuclear receptor ligands that mitigate triphenyl phosphate-induced cardiotoxicity in zebrafish embryos

Constance Mitchell[1] and David Volz[2]

[1]Environmental Toxicology, University of California Riverside, Riverside, CA, United States; [2]Environmental Science, University of California Riverside, Riverside, CA, United States
cmitc007@ucr.edu

Triphenyl phosphate (TPHP) is a high-production volume organophosphate flame retardant within the United States. We previously showed that embryonic exposure to TPHP results in cardiac looping impairments. The objective of this study was to rely on high-content screening to identify nuclear receptor ligands that enhanced or mitigated TPHP-induced cardiotoxicity during zebrafish embryogenesis. Embryos were exposed from 24 to 72 hours post fertilization (hpf) under static conditions to vehicle or cardiotoxic concentrations of TPHP in the presence or absence of 74 different nuclear receptor ligands. Hatched and alive 72-hpf embryos were analyzed for body length and pericardial area, a biomarker for cardiac looping defects. We identified two compounds (ciglitazone and fenretinide) that mitigated TPHP-induced cardiotoxicity. These data suggest that TPHP interferes with PPARγ- and RAR-mediated signaling pathways, as ciglitazone and fenretinide are agonists for these receptors, respectively.

VII-234

Bridging the gap from external to internal exposure: Use of *in silico*, PBPK and *in vitro* methods to predict the biokinetics of topically applied cosmetic ingredients

Martina Klaric[1], Richard Cubberley[2], Hélène Duplan[3], Joan Eilstein[4], Corie Ellison[5], Sebastien Grégoire[4], Nicky Hewitt[1], Carine Jacques-Jamin[3], Daniela Lange[6], Amy Roe[5], Helga Rothe[7] and Andreas Schepky[6]

[1]Cosmetics Europe, Brussels, Belgium; [2]Unilever, Sharnbrook, United Kingdom; [3]Pierre Fabre, Toulouse, France; [4]L'Oréal, Aulnay sous Bois, France; [5]Procter and Gamble, Cincinnati, OH, United States; [6]Beiersdorf AG, Hamburg, Germany; [7]Coty, Darmstadt, Germany
mklaric@cosmeticseurope.eu

The estimation of internal exposure of a topically applied chemical from the skin surface across the skin layers and into the systemic circulation is a key step in the assessment of potential toxic effects. Input data from *in vitro* assays can be applied to physiological based pharmacokinetic (PBPK) models which are used to estimate the absorption, distribution, metabolism and excretion (ADME) of chemicals. Once an internal concentration is estimated, it can be related to known concentrations causing toxicity. Following on from this, the link between the internal exposure and the No Adverse Effect Levels (NOAELs) can then be used to develop the internal Threshold of Toxicological Concern (TTC) concept. The concept of predicting the internal exposure with only alternative methods and developing the internal TTC concept are a key part of the CE Long Range Science Strategy.

VII-239

The study of technical transferability and inter-laboratory reproducibility of the SH test (Part 2)

Noriyasu Imai[1], Sakiko Aizawa[1], Midori Takeyoshi[1], Kaoru Kasahara[2], Maki Sugiyama[2], Akemi Toyoda[2] and Morihiko Hirota[3]

[1]KOSÉ Corporation, Tokyo, Japan; [2]POLA Chemical Industries, Inc., Yokohama, Japan; [3]Shiseido Global Innovation Center, Yokohama, Japan

n-imai@kose.co.jp

Development of alternative methods is an urgent issue for the safety assessment of cosmetic products and ingredients. The SH test (Hirota et al., 2013) is one of the alternative methods for skin sensitization, detecting the changes of cell-surface thiols by the treatment with haptens. We have evaluated technical transferability of the SH test among three laboratories since 2015 (Takeyoshi et al., 2015). The aim of this study was to promote efficiency and rationalization of the SH test.

The SH test was performed according to the refined protocol based on the latest studies (Takeyoshi et al., 2015, 2016; Sugiyama et al., 2016). First, we revised the evaluation flowchart. Then we evaluated 25 chemicals by the refined protocol and new flowchart. The obtained results were compared to the published *in vivo* data.

The refined protocol and the new flowchart improved the inter-laboratory reproducibility and experimental efficiency with high concordance rate to the published data. Consequently, the refinement can improve the availability of the SH test for evaluation of skin sensitization.

References

Hirota, M., Kouzuki, H., Ashikaga, T. et al. (2013). Artificial neural network analysis of data from multiple in vitro assays for prediction of skin sensitization potency of chemicals. *Toxicol In Vitro 27*, 1233-1246.

Sugiyama, M. et al. (2016). Japanese Society of Toxicology 43rd Annual Meeting.

Takeyoshi, M. et al. (2015). JSAAE 28th Annual Meeting.

Takeyoshi, M. et al. (2016). JSAAE 29th Annual Meeting.

III-321

Chemically defined cell culture practice of L929 fibroblasts

Roman Kolar[1], Joachim Wiest[2], Michael Feil[2] and Tilo Weber[1]

[1]German Animal Welfare Federation – Animal Welfare Academy, Neubiberg, Germany; [2]cellasys GmbH, Kronburg, Germany

Roman.Kolar@tierschutzakademie.de

Living cells are a promising tool to develop alternatives to animal experiments. To provide them for cell based assays e.g. cell lines are kept in CO_2 incubators and split once a week with feeding approximately every third day. The guidelines for good cell culture practice give recommendations how to work in cell culture (Coeke et al., 2005). A remaining issue is that many laboratories use fetal bovine serum (FBS) in standard cell culture. Beside the questionable way of manufacturing of FBS (van der Valk et al., 2010), it is chemically not defined. The variations in FBS may be a driver for the reproducibility problem in biomedical research. For our L929 cell line, the cell culture media with 10% FBS was replaced by the chemically defined DME/F12 + ITS mixture (van der Valk et al., 2010). With this procedure, we maintained the cell line now for more than a year. Although the cells changed their morphology they are used successful in biocompatibility testing (Wiest, 2017) and in microphysiometric experiments to determine the eye irritation potential of chemicals.

References

Coecke, S., Balls, M., Bowe, G. et al. (2005). Second ECVAM task force on good cell culture practice. Guidance on good cell culture practice. a report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim 33*, 261-287.

van der Valk, J., Brunner, D., De Smet, K. et al. (2010). Optimization of chemically defined cell culture media – Replacing fetal bovine serum in mammalian in vitro methods. *Toxicol In Vitro 24*, 1053-1063.

Wiest, J. (2017). Chemisch definiert – Ein zellbasierter Zytotoxizitätsassay ohne fötales Kälberserum. *Biospektrum 23*, 61.

VII-327

Guidance for industry on alternatives for chemical and cosmetic testing

Katy Taylor

Science and Regulatory Affairs, Cruelty Free International, London, United Kingdom

katy.taylor@crueltyfreeinternational.org

There are several alternatives to animal testing now available to cosmetic and chemical companies. However, understanding which methods are acceptable to the relevant regulator is often confusing, particularly for SMEs. To assist these industries, Cruelty Free International has produced two separate guides. "How to avoid new animal tests in your 2018 REACH registration" assists SMEs and REACH consultants in identifying the recent changes to OECD Test Guidelines and the REACH legislation. Important updates are the removal of the rabbit skin and eye irritation tests, the option to avoid the dermal acute toxicity test and the need to test for skin sensitisation *in vitro* to identify non- sensitisers. "Meeting the Global Challenge: A guide to assessing the safety of cosmetics without using animals", provides a similar update for cosmetic companies as part of our worldwide campaign to end cosmetics testing.

VII-333

Development of novel Threshold of Toxicological Concern (TTC) for botanical extracts

Taisuke Kawamoto[1], Anne Fuchs[1], Osamu Morita[2] and Rolf Fautz[1]

[1]Safety & Regulatory, Kao Germany, Darmstadt, Germany; [2]Safety Science Research, Kao Corporation, Ichikai, Japan
Taisuke.Kawamoto@kao.com

Threshold of Toxicological Concern (TTC) is one of the promising exposure-based approaches to evaluate potential risks for systemic toxicity without animal testing. This concept has been developed and validated for single substance, not for the chemical mixture. Thus, the applicability for the mixture is still unclear.

From literature-based survey including over 187 papers, over 225 No-Observed-Adverse-Effect-Level (NOAEL) were corrected by available literature in botanical/plant/herbal extracts. These NOAELs were divided by uncertainty factor (UF) of 100 used for cosmetics. In addition, we multiplied additional safety factor depending on the test conditions (i.e. using mouse: 1.75, < 90 days: 3). Comparing the lowest 5 percentile value derived from adjusted NOAEL (as novel TTC) and classical Cramer III-TTC value, novel TTC was adequately conservative. This result indicates that the risk for systemic toxicity is negligible if the exposure is below 90 mg/day. This abstract does not reflect the policy of Kao Corporation.

VII-351

The bicycle ergometer test: A human stress model to test nutritional effects on intestinal function and immune responsiveness

Cyrille Krul[1], Shirley Kartaram[1], Marco Mensink[2], Marc Teunis[1], Eric Schoen[3], Martie Verschuren[4], Laura M'Rabet[1], Klaske Van Norren[2], Johan Garssen[5], Renger Witkamp[2] and Raymond Pieters[1]

[1]University of Applied Sciences Utrecht, Utrecht, The Netherlands; [2]Wageningen University & Research Centre, Wageningen, The Netherlands; [3]TNO, Zeist, The Netherlands; [4]Avans University of Applied Sciences, 's-Hertogenbosch, The Netherlands; [5]UIPS, Utrecht University, Utrecht, The Netherlands
cyrille.krul@hu.nl

The bicycle ergometer test may be useful to support human nutritional claims, but the test has not been optimized and properly validated yet. We therefore aim to determine kinetic changes in intestinal function and immune responsiveness in relation to extent of exercise.

Fifteen healthy young men cycled 1 h at different W_{max} intensities: 70% in (de)hydrated condition, 85/55% and 50%. Blood samples were collected at several timepoints up to 24 h. Biomarkers of intestinal function, immune responsiveness and general physiology were measured. Data was analyzed using a multilevel mixed linear statistical model, and shows that immune responsiveness (e.g. NK cells, neutrophils), intestinal function (e.g. intestinal fatty acid binding protein) and general physiology (e.g. cortisol) changed kinetically peaking between 0 and 6 hrs.

Importantly, kinetic changes were also observed at low exercise intensity, allowing to test less healthy individuals in the future as well. Altogether, we aim to use this human stress model as alternative model to investigate nutritional health claims.

VII-352

VitalTissue.nl: A fresh human tissue supply chain to enable translational research

Cyrille Krul[1], Martje Fentener van Vlissingen[2], Gwendolynn Grootaers[3], Geny Groothuis[4], Joost van Hilten[5], Evita van de Steeg[6], Evert Ben van Veen[7] and Beppy van de Waart[8]

[1]University of Applied Sciences Utrecht, Utrecht, The Netherlands; [2]Erasmus MC, Rotterdam, The Netherlands; [3]Toxys, Leiden, The Netherlands; [4]University Groningen, Groningen, The Netherlands; [5]Sanquin, Amsterdam, The Netherlands; [6]TNO, Zeist, The Netherlands; [7]MedLawConsult, Den Haag, The Netherlands; [8]Charles River, Den Bosch, The Netherlands
cyrille.krul@hu.nl

Currently used cell lines and animal models lack translational value to the human situation. The need for vital human tissue is clear, however the problem is limited availability and accessibility, especially for non-academic and researchers within industry. There is no organized infrastructure for fresh human tissue supply. VitalTissue.nl is an online platform for suppliers and users of viable, non-frozen human tissue. VitalTissue.nl will provide accessibility and transport of vital human tissue to all researchers. It is an independent, not-for profit organization on a national level, that can act rapidly in response to regional demands, supplementing biobanks. Involvement of all relevant stakeholders, especially patients will be organized and privacy and security of personal data must be guaranteed. A feasibility study is started investigating these aspects and to develop a sustainable business model. Based on stakeholder interviews, quality and logistics standards, ethical and legal implications are considered. This concept will enable better translational models, faster drug development and contribute to the reduction of animal testing.

VII-391

Heat-killed *Malassezia pachydermatis* modulates macrophage activity against *Encephalitozoon cuniculi* infection *in vitro*

Maria Anete Lallo[1], Jessica Feliciana Cocedeira[1], Fabiana Toshie de Camargo Konno[1], Elizabeth Christina Perez[1], Diva Denelle Spadacci-Morena[2], Selene Dall Acqua Coutinho[1] and Anuska Marcelino Alvares-Saraiva[1]

[1]Environmental and Experimental Pathology, Paulista University, Brasília, DF, Brazil; [2]Butantan Institute, Butantã, SP, Brazil
anetelallo@hotmail.com

Encephalitozoon (E.) cuniculi is an opportunistic agent which causes infectious diseases both in humans and other animals. *Malassezia (M.)* spp. are commensal microorganisms able to modulate pro and anti-inflammatory cytokines production by keratinocytes. Here, we evaluated the heat-killed *M. pachydermatis* ability to regulate macrophages against *E. cuniculi* infection. Treated-macrophages showed 70 to 95% of *E. cuniculi* spores phagocytosis with phagocytic index range of 3 to 5,5. Non-treated macrophages showed 20 to 40% phagocytosis percentual and phagocytic index lower than 1. On the other hand, heat-killed *M. pachydermatis* treatment down regulates the nitric oxide (NO) production by macrophages while *E. cuniculi* infection increases NO levels. Together, results revealed that *M. pachydermatis* previous treatment regulates controversially macrophage activity, increasing phagocytosis ability but decreasing nitric oxide production.

References

Kesavan, S., Holland, K. T. and Ingham, E. (2000). The effects of lipid extraction on the immunomodulatory activity of Malassezia species in vitro. *Med Mycol 38*, 239-247.

Valencakova, A. and Halanova, M. (2012). Immune response to Encephalitozoon infection review. *Comp Immunol Microbiol Infect Dis 35*, 1-7.

VII-392

Blue LED therapy reduces phagocytic capacity of murine macrophages

Maria Anete Lallo, Brenda Camargo Camargo, José Renildo de Carvalho, Débora Silvestro, Fernanda Pereira Belo Cavalcante, Thiago Albuquerque and Elizabeth Christina Perez

Environmental and Experimental Pathology, Paulista University, Brasília, DF, Brazil
anetelallo@hotmail.com

The use of phototherapy with LED was shown to be an effective treatment for various dermatological diseases for promoting satisfactory wound healing. Since macrophages are involved in the healing process, the aim of this work was to evaluate *in vitro* the effects of LED phototherapy on the phagocytic capacity of murine macrophages. For this, RAW 264.7 macrophages were treated with blue or red LED for 30 minutes. The heat inactivated bread yeast particles were added at a 1:10 effector target ratio and incubated for two hours. Coverslips were collected at 0, 6, 24 and 48 hours and submitted to Giemsa stained to calculate phagocytic index. Results of light microscope analyses revealed that macrophages treated with blue LED presenting less number of internalized yeast when compared to macrophages treated with red LED or untreated. This preliminary result indicates that in an *in vitro* model, blue LED therapy reduces of phagocytic capacity of murine macrophages.

References

Frangez, I., Cankar, K., Ban Frangez, H. and Smrke, D. M. (2017). The effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients-a prospective, double-blind randomized study. *Lasers Med Sci 32*, 887-894. doi:10.1007/s10103-017-2189-7

Hadis, M. A., Cooper, P. R., Milward, M. R. et al. (2017). Development and application of LED arrays for use in phototherapy research. *J Biophotonics*, Epub ahead of print. doi:10.1002/jbio.201600273

VII-398

Description of a pro-fibrotic biomarker pattern in *ex vivo* lung tissue slices with high clinical relevance

Christina Hesse[1], Samuel Mang[2], Heinz-Gerd Hoymann[1], Monika Niehof[1], Peter Braubach[3], Danny Jonigk[3], Mark Kühnel[3], Gregor Warnecke[3], Olaf Pfennig[4], Hans-Gerd Fieguth[4], Armin Braun[1] and Katherina Sewald[1]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine, Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [2]Boehringer Ingelheim Pharma GmbH and Co. KG, Immunology and Respiratory Diseases Research, Biberach, Germany; [3]Hannover Medical School, Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [4]KRH Clinics, Hannover, Germany
christina.hesse@item.fraunhofer.de

Pulmonary fibrosis covers a scope of rapidly progressing lung diseases, resulting in irreversible pulmonary dysfunction. Current animal models do not adequately reflect all features of these diseases, which further hampers the development of new therapies. Precision-cut lung slices (PCLS)provide a physiologically relevant model to investigate different aspects of pulmonary fibrosis *ex vivo*.

PCLS prepared from lungs of bleomycin-treated rats retain the pro-fibrotic mRNA pattern in culture for up to 5 days. Fn1 or col1a1were > 2-fold elevated compared to control slices. Human PCLS stimulated with TGFβ and TNFα revealed a comparable pattern. In addition, important pro-fibrotic mediators, e.g. PAI1, were significantly elevated. IL1β levels were significantly elevated in both experimental *ex vivo* systems.

We describe here a novel pattern of pro-fibrotic biomarker in PCLS in two different experimental setups that allow the investigation of pulmonary fibrosis with high translational relevance.

III-420

Properly designed controls play an essential part in achieving the promise of precision for *in vitro* bioassays

John Harbell[1] *and Peggy Cunniff*[2]

[1]JHarbell Consulting LLC, Dallas, TX, United States; [2]National Anti-Vivisection Society/International Foundation for Ethical Research, Chicago, IL, United States
johnharbell@sbcglobal.net

One promise of *in vitro* bioassays (i.e., toxicology) is greatly increased precision relative to the *in vivo* methods they replace. This promise is predicated, in part, on greater control of the test system, exposure conditions and endpoint measures. It is also based on the ability to design and perform the appropriate controls for each assay to verify the integrity of the test system and proper assay execution. Responsibility falls to the developer for designing the proper controls for the assay and to the user for consistently running those controls. Most *in vitro* bioassays are based on a quantitative response which is used to predict the toxicological or other effect in man. Hyper or hypo sensitivity of an assay trial (invalidating the prediction model) must be identified so that spurious predictions for the unknown test materials are avoided. This presentation will provide guidance on designing controls and examples of the consequences when those controls were not performed.

VII-433

Development of a thyroid neurovascular unit organotypic systems model

Andrew Schwab and Sidney Hunter

U.S. EPA, Research Triangle Park, NC, United States
schwab.andrew@epa.gov

During human development, disruption of thyroid hormone (TH) signaling is related to adverse morphological effects including altered brain development and function. The quantitative relationship between mild and moderate levels of TH disruption on neural morphogenesis, particularly in the face of xenobiotic challenge, remains unknown. We focus on the human neurovascular unit (NVU) and early nervous system development using dynamic (ibidi 2-compartment microfluidic slide) and static (transwell) models comprised of endothelial, pericyte, and astrocyte and neuron progenitor cells. We demonstrate low permeability across the NVU/blood-brain barrier as well as glio- and neuro-genesis of EZ Sphere stem cell-derived neural progenitors. Future studies to evaluate the morphological effects of thyroid hormone disrupting chemicals will allow a determination of the consequences of hypothyroidism or TH signaling disruption during brain development.

This abstract does not represent U.S. EPA policy.

VII-607

Cigarette smoke induces biomarkers of COPD in fresh human lung tissue

Helena Obernolte[1]*, Detlef Ritter*[1]*, Jan Knebel*[1]*, Peter Braubach*[2]*, Danny Jonigk*[2]*, Gregor Warnecke*[2]*, Patrick Zardo*[2]*, Hans-Gerd Fieguth*[3]*, Olaf Pfennig*[3]*, Armin Braun*[1] *and Katherina Sewald*[1]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine, Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [2]Medical School Hannover, Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany; [3]KRH Clinics, Hannover, Germany
helena.obernolte@item.fraunhofer.de

Cigarette smoke (Cs) inhalation is a main reason to develop chronic obstructive pulmonary disease (COPD). We assessed the effects of Cs and Cs condensate (Csc) on fresh human lung tissue.

Human Precision-Cut Lung Slices (PCLS) were exposed to Csc or whole Cs in an Air-Liquid Interface (ALI) using *in vitro* exposure device P.R.I.T.® ExpoCube®. Tissue viability, release of cytokines and extracellular matrix (ECM) proteins were analysed. Inhibitors were applied to suppress inflammatory responses of tissue to Cs. Csc and Cs induced concentration-dependent cytotoxicity in fresh lung tissue. Csc and Cs exposure induced an increased release of pro-inflammatory cytokines IL-1α, IL-1β and changes in ECM proteins, e.g. MMP-9 and pro-Collagen1α1, from PCLS. Dexamethasone and Roflumilast inhibited CS-induced increase of pro-inflammatory cytokines.

Csc and Cs induced tissue damage and early biomarkers of inflammation and changes in ECM proteins in vital *ex vivo* human lung tissue.

VII-751

Next steps towards responsible animal-based research

Marlies Leenaars, Judith van Luijk and Merel Ritskes-Hoitinga

Health Evidence, SYRCLE Radboudumc, Nijmegen, The Netherlands

marlies.leenaars@radboudumc.nl

Animal models are being used based on the assumption that the findings from animal studies can be translated to humans. However, when animal studies fail to translate, animals may have been used ineffectively or unnecessary. In this project, we have evaluated the implementation of the 3R-principle (Replacement, Reduction and Refinement of animal studies) in the Netherlands. Additionally, a novel method namely – systematic reviews of animal studies – was introduced and evaluated as a new strategy to improve responsible animal use in research. Systematic reviews are a transparent method to summarise and re-analyse available data in order to gain new insights. The project concludes with four practical recommendations on how the systematic review methodology can contribute to responsible and high-quality animal-based research.

Reference

Full thesis: http://www.digitaalproefschrift.nl/ebooks/Judith%20Van%20Luijk%20thesis%20eBook/mobile/index.html#p=1

VII-771

An imaging assay to analyze primary hippocampal neurons for cellular maturation

Tomoaki Shirao[1], Kenji Hanamura[1], Noriko Koganezawa[1], Hiroyuki Yamazaki[1] and Yuko Sekino[2]

[1]Dept Neurobiology & Behavior, Gunma University Graduate School of Medicine, Maebashi, Japan; [2]Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan

tshirao@gunma-u.ac.jp

Evaluation of the status of synapses is more difficult than the quantification of cell number and dendrite length of neurons. In this study, we used drebrin as a marker of synapse maturation. Frozen stock of hippocampal neurons was used to minimize the variation among experiments. After 21 days *in vitro*, neurons were fixed and processed for immunocytochemistry to visualize drebrin and MAP2 with nuclear staining. After automated image acquisition, mature synapse density along dendrites, dendritic length and neuronal number were automatically evaluated by using newly-developed algorism. We found that the dendrite growth and spine formation are differentially regulated. The dose-dependent glutamate effect on drebrin clusters was reproducibly detected without affecting neuronal number and dendritic length. This effect was inhibited by NMDA receptor antagonist. These results suggest that this high-content analysis for synapses will be useful for detecting the effect of drugs that may affect synapse formation and function as well as neuronal maturation.

VII-804

Serum-free media and serum alternatives

Jan van der Valk[1], Roman Kolar[2] and Gerhard Gstraunthaler[3]

[1]3Rs-Centre Utrecht Life Sciences, Utrecht, The Netherlands; [2]Animal Welfare Academy, German Animal Welfare Federation, Neubiberg, Germany; [3]Division of Physiology, Medical University Innsbruck, Innsbruck, Austria

janvdrvalk@gmail.com

The use of foetal calf serum (FCS) in the biosciences has been regarded critically for decades. From a scientific point of view, the use of undefined media supplements such as FCS is problematic for a range of applications, e.g. when ingredients mask the toxic effect of substances which bind to them. From an ethical perspective, the production of FCS is connected to various serious animal welfare problems because it involves the heart puncture of live foetuses. Nevertheless, to date FCS is used at large scale, particularly for cell cultures.

This presentation reports on the outcome of a 3rd workshop on this topic, held in June 2016, which brought together key players in the field, to connect to previous activities and investigate solutions for the future.

The 1st workshop, held in Utrecht, The Netherlands, in 2003 (van der Valk et al., 2004) was initiated to create awareness and to discuss possibilities to reduce or replace the use of FBS in cell culture media. A follow-up workshop was organized in Copenhagen, Denmark, to discuss current *in vitro* methods devoid of FBS or other animal components (van der Valk et al., 2010).

A 3rd workshop on FBS, serum alternatives and serum-free media was organised in light of new developments. Three main topics were identified to discuss at this workshop: (1) the serum controversy, (2) alternatives to FBS, with special emphasis on human platelet lysates, databases on serum-free media, commercialization of chemically defined media, and (3) serum-free *in vitro* applications.

Theme VIII – Refinement and Animal Welfare

Coordinators
Greg Reinhard, MBA, DVM, DACLAM, University of Pennsylvania, Philadelphia, PA, United States
Patricia V. Turner, MS, DVM, DVSc, DACLAM, DABT, DECAWBM (WSEL), University of Guelph, Guelph, ON, Canada
Thierry Decelle, Sanofi, Paris, France

Session VIII-1: Oral Presentations

Session VIII-1: Evolution of Research Animal Welfare

Co-Chairs
Susanna Louhimies, DG Environment, European Commission, Brussels, Belgium
Gilly Griffin, Canadian Council on Animal Care, Nottingham University, Ottawa, Canada

VIII-1-681

Evolution of refinement – From concept to practice

Susanna Louhimies[1] and David B. Anderson[2]

[1]DG Environment, European Commission, Brussels, Belgium; [2]European Commission / PMS, Brussels, Belgium
susanna.louhimies@ec.europa.eu

The Three Rs of Russell and Burch have provided a solid foundation for the current legislative framework for animal use and care in Europe.

With time, Refinement has evolved from its original consideration during animal use to an all-encompassing concept to be taken into account before, during, in between and after experiments; a continuous Refinement is a legal obligation during use as well as in all breeding and care practices.

The talk will discuss the key elements and infrastructures that provide the right setting to implement a continued Refinement in all interaction with animals. The developed Severity Assessment Framework Guidance provides tools on how to consider Refinement from the project design to day-to-day application and follow-up. The guidance for Animal Welfare Bodies and National Committees provide further ideas on how to build up the necessary support structures enabling animal welfare to remain a central focus in all care and use of animals.

VIII-1-548

Between evidence base and speciesism – A brief history of laboratory fish welfare

Paul Schroeder

Dept of Biomedical Services, University of Oxford, Oxford, United Kingdom
paul.schroeder@apha.gsi.gov.uk

Early milestones in the advancement of clinical fish welfare and welfare science include the first use of MS 222 by R. Schoettgerat the Wisconsin Sports Fishing Laboratory in the 1960s and the behavioural assays on carp by J. Verheijen in the Netherlands in the 1970s and 80s. The latter remained relatively obscure but re-emerged in the 1990s and 2000s after the publication of studies by L. Sneddon, F. Huntingford, V. Braithwaite and others linking new findings on behavioural ecology of fish with are appraisal of their welfare needs. The resulting shift in the perception offish sentience has triggered a perennial debate on the capacity of fishes for higher conscience with the detractors mainly quoting an antiquated speciesist paradigm.

Over the last five years new advances have come through on the aversiveness of common fish anaesthetics, on utility of environmental enrichment and, more recently, the use of immersion analgesics in fish procedures. Ongoing studies explore anaesthetic efficacy and new setups incorporating sedative pre-medication. It is hoped that the knowledge gained through the laboratory refinement drive will benefit fish welfare in all areas.

VIII-1-20

Focussing on severe suffering

Elliot Lilley, Penny Hawkins and Barney Reed

Research Animals, Royal Society for the Prevention of Cruelty to Animals, Horsham, United Kingdom
elliot.lilley@rspca.org.uk

In 2010, the RSPCA began working with the scientific community to develop and promote new approaches to help reduce the number of animals experiencing the highest level of suffering in research and testing (e.g. USDA category E).
As a scientific animal welfare organisation with a high level of liaison with scientific and regulatory communities, we have established a well-supported programme of work that has to date included:
– A comprehensive web resource to help the research community address severe suffering: www.rspca.org.uk/severesuffering.
– Downloadable guidance to help establishments through the process of reducing severe suffering.
– Five expert working group reports on reducing suffering in specific procedures, e.g. sepsis and rheumatoid arthritis.
– An International "Focus on severe suffering" meeting in Brussels in 2016.

This talk will provide more information on our severe suffering resources and explain how we work with the scientific community.

References

Hawkins et al. (submitted). Applying refinement to the use of mice and rats in rheumatoid arthritis research.
Lilley et al. (2014). A 'road map' toward ending severe suffering of animals used in research and testing. *Altern Lab Anim 42*, 267-272.
Lilley et al. (2015). Refinement of animal models of sepsis and septic shock. *Shock 43*, 304-316.
Wolfensohn et al. (2013). Reducing suffering in animal models and procedures involving seizures, convulsions and epilepsy. *J Pharmacol Toxicol Methods 67*, 9-15.
Wolfensohn et al. (2013). Reducing suffering in experimental autoimmune encephalomyelitis (EAE). *J Pharmacol Toxicol Methods 67*, 169-176.

VIII-1-249

Establishment of a Global Pharmacology Council to optimize and standardize *in vivo* models and secure full integration of the 3Rs in decision making processes in a global research unit

Thora B. Bödvarsdottir

3R Strategy & Veterinary Services, Global Research, Novo Nordisk A/S, Maaloev, Denmark
tora@novonordisk.com

At Novo Nordisk we have 3 global sites that work with *in vivo* pharmacology.

In order to ensure cross site quality and reproducibility, we have established a Global Pharmacology Council (GPC) which reports to the Global Research management team.

The GPC has governance of all *in vivo* models and technologies (model catalogue) used to create data for milestone passages of new drug candidates. The council is responsible for ensuring global quality and reproducibility and drive that learnings are shared globally. Furthermore we strive to have all models assigned to a centre of excellence to ensure state of the art animal research.

All new models have to be approved by the GPC which evaluates the unmet need, scientific rationale, translational value and the 3Rs. The GPC guides in the establishment and characterization of a model including definition of success criteria. With a positive conclusion on a new model, the model enters the model catalogue.

Learnings from the past 2 years, especially from a 3R perspective, will be presented and discussed.

VIII-1-531

Rehoming of laboratory animals: Local policy and dilemmas in an academic setting

Wim Leeuw and Harry Blom

Animal Welfare Body, Utrecht University, Utrecht, The Netherlands
w.a.deleeuw@uu.nl

Directive 2010/63/EU contains provisions for the rehoming of former laboratory animals and the conditions under which this can occur. These terms and conditions are incorporated in the Dutch Experiments on Animals Act (Wod). For animals that have been used or were intended for use in an animal procedure rehoming can be an option provided the condition the animal allows it, there is no danger to public health, animal health or the environment and when appropriate measures have been taken to ensure the welfare of the animal. In 2016, the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) has issued an Opinion on the rehoming of laboratory animals as well as specific Codes of Practice on the rehoming of cats, dogs and non-human primates.

Although Utrecht University and the University Medical Centre Utrecht (UMC Utrecht) had a rehoming policy already for years, this momentum was used to critically review the existing local policy. UU and UMC Utrecht not only house many different animal species, but also animals with a different status: in addition to laboratory animals also animal patients and animals used for farming are housed, often in the same facilities. Many of these are taken care of by the same professionals. Different legal regimes apply to these categories of animals. This can result in different conditions for housing, care and monitoring.

Especially in cases when a choice has to be made between re-use, euthanasia, or rehoming of animals these differences often give rise to profound discussions between students, animal care takers, veterinarians and members of the AWB

In this presentation, an outline is given of the UU/UMC Utrecht policy on rehoming of animals. The starting point for UU and UMC Utrecht is that for surplus laboratory animals rehoming should be considered as a serious option. In project proposals and work protocols, applicants should indicate whether rehoming is possible and provide a reason when stating that it is not. The policy of UU/UMC Utrecht aims at creating conditions that foster a positive attitude and make rehoming easier and more successful. At the same time, it should provide for a structured and transparent framework, including a set of preconditions, which have to be clear and discussed before an experiment starts that includes animals for which rehoming may be an option. For instance, it must be clear already before an experiment starts how responsibilities are organized and how early socialisation is realized. This facilitates the rehoming process at a later stage. At the same time it is also advantageous for the establishments themselves: socialised animals are less stressed and more manageable, which is beneficial to the quality of the research. The premise is that rehoming should always be in the interest of the welfare of the animal.

The role of the Animal Welfare Body and other experts is illustrated. On the basis of some cases, special attention is given to the category of animals with an ailment. This is where differences in legal regimes, in ethical views and in professional visions are felt the most.

Session VIII-2: Advances in Technology to Enhance Animal Welfare

Co-Chairs
Thierry Decelle, Sanofi, Paris, France
Paul Schroeder, Animal & Plant Health Agency, Addlestone, United Kingdom

VIII-2-731

Bringing behavioral management together with instrumentation, a transformative technology for animal-centric care

Melanie Graham
University of Minnesota, Saint Paul, MN, United States
graha066@umn.edu

The combination of instrumentation and behavioral management provides a transformative technology which presents a broadly accessible tool for caregivers to positively impact the animal experience. Behavioral management programs for animals are well-established and essential in providing opportunities to express species typical behaviors as well as circumstances to make choices or exercise control over their environment. NHPs can be trained using positive reinforcement to voluntarily cooperate with necessary clinical care. Likewise, invasive and or stressful handling can be limited by expanding training to incorporate instrumentation. Medical devices designed to reduce burden to patients have demonstrated meaningful improvements in health-related quality of life and are often adaptable for animal-centric application. The deliberate merging of technologies improves animal well-being and maximizes the likelihood of accurate translation of experimental data to the clinical condition.

VIII-2-116

Refining dog care: Evidence-based refinements to improve dog welfare and data output

Laura Scullion Hall and Hannah Buchanan-Smith
Psychology, University of Stirling, Stirling, United Kingdom
laura.hall@stir.ac.uk

The dog is the most commonly used non-rodent species in the safety assessment of new chemical entities (> 100,000 pa) yet we know little about their welfare and impact of routine practices on welfare (Prescott et al., 2004). While there is broad desire to implement effective Refinements to many aspects of dog use there are barriers to uptake, including lack of evidence and resources specific to the research environment, and concerns about interference with data quality and study outputs. From our collaborative project across UK industry (http://www.refiningdogcare.com), we present evidence-based resources for good practice and a number of protocols will be shared. Techniques to improve welfare and data output, and prepare dogs for study life will be presented. These include facility and home pen design (Scullion Hall et al., 2017), enrichment (Hall, 2014), training (Scullion Hall and Robinson, 2016), predictability (Scullion Hall et al., submitted), handling and dosing techniques (Hall et al., 2015). We describe empirical evidence demonstrating both welfare benefits and ease of implementation of an effective training protocol for laboratory-housed dogs are described. Our welfare assessment framework (Hall et al., 2015) is employed to monitor the impact of planned Refinements on welfare, and to evaluate preparation for procedures.

References

Hall, L. E. (2014). A practical framework for harmonising welfare and quality of data output in the laboratory-housed dog. Unpublished thesis. University of Stirling, Stirling, UK.

Hall, L. E., Robinson, S. and Buchanan-Smith, H. M. (2015). Refining dosing by oral gavage in the dog: A protocol to harmonise welfare. *J Pharmacol Toxicol Methods 72*, 35-46.

Prescott, M. J., Morton, D. B., Anderson, D. et al. (2004). Refining dog husbandry and care. *Lab Anim 38*, 96.

Scullion Hall, L. E. M. and Robinson, S. (2016). Implementing a successful positive reinforcement training protocol in laboratory-housed dogs. *Animal Technology and Welfare 15*.

Scullion Hall, L. E. M., Robinson, S., Finch, J., and Buchanan-Smith, H. M. (2017). The influence of facility and home pen design on the welfare of the laboratory-housed dog. *J Pharmacol Toxicol Methods, 83*, 21-29.

Scullion Hall, L. E. M., Robinson, S., Finch, J. and Buchanan-Smith, H. M. (submitted). Anticipation and predictability as tools to improve welfare in laboratory-housed dogs.

VIII-2-69

Dosage matters – Tramadol applied via the drinking water for pain management in bone-linked mice models

Annemarie Lang[1,2,3], Mattea Durst[1], Frank Buttgereit[1,3], Katharina Schmidt-Bleek[4] and Paulin Jirkof[5]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Berlin-Brandenburg School of Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany; [3]German Rheumatism Research Centre, Berlin, Germany; [4]Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Charité-Universitätsmedizin Berlin, Berlin, Germany; [5]Division of Surgical Research, University Hospital Zurich, Zurich, Switzerland

annemarie.lang@charite.de

An alternative to repeated injections is the application of analgesics via the drinking water. However, studies that address the efficiency of Tramdol in the drinking water are scarce. Different recommendations exist regarding the dosage, and they widely differ from potentially under- to overdosing. We performed a refinement study embedded in a basic research study in the mouse osteotomy model evaluating two commonly used pain management protocols, Tramadol (two concentrations) and Buprenorphin in the drinking water, for their efficiency and side effects on experimental readout in a mouse osteotomy model. We monitored (i) general parameters of wellbeing e.g. MGS, clinical scoring, weight, water, food uptake and (ii) model specific pain parameters. Our results show that high dosage of Tramadol can lead to sedation and reduced wellbeing compared to an effective but lower dose or buprenorphine treatment. No side effects on bone healing read-outs (μCT, histology) occurred.

VIII-2-524

Development of non-lethal sampling methodology to investigate salmonid host immune responses to ectoparasites

Rachel Chance[1,2], Christopher Secombes[1], Catherine Collins[2] and Bertrand Collet[2]

[1]Scottish Fish Immunology Research Centre, Aberdeen, United Kingdom; [2]Marine Laboratory, Marine Scotland Science, Aberdeen, United Kingdom

r01rc14@abdn.ac.uk

Recently fish immunologists developed approaches for individual fish monitoring to reduce fish use, especially in disease studies. To date the focus has been viral and bacterial pathogens but many health issues are due to ectoparasites. Hence, we have established an individual monitoring methodology for amoebic gill disease, caused by the parasite *Neoparamoeba perurans*. To ensure data from individually monitored fish are representative of natural disease progression, the effect of repeated anaesthesia on both parasite and host was assessed. After selecting AQUI-S® as appropriate anaesthetic, post-smolt salmon were PIT-tagged and challenged (2500 amoeba/L^{-1}). Comparative analysis of gill scores confirms that repeated gill swabs do not alter disease progression. Gene expression of non-lethal samples mirror upregulation in lethally obtained tissues. In summary these samples convey immunologically valid data, highlighting the potential of improving on 3R's in aquaculture disease research.

VIII-2-76

Refinement of repeated arterial blood sampling in pigs

Vladimir Bubalo, Iris Wiederstein-Grasser and Birgit Reininger-Gutmann

Medical University of Graz, Division of Biomedical Research, Graz, Austria

vladimir.bubalo@klinikum-graz.at

Preclinical *in vivo* studies continuously testing glucose sensors over a longer period of time require frequent blood sampling to verify the sensor readings. Repeated arterial blood sampling in pigs is challenging due to their predisposition to vasospasm and vessel rupture (Smith and Swindle, 2008; Wolfensohn and Lioyd, 2006). The cannulation of the saphenous artery, which is routinely used during anaesthesia, does not provide safe access for frequent collection of bigger blood amounts, because of the smaller diameter of the catheter and risk of collapse of the vessel. In order to improve the ease and quality of frequent blood sampling and to further increase the number of sensors simultaneously tested per pig, a four-sided central venous catheter was placed into the A. carotis communis and Arteria femoralis sinistra et dextra. This method allowed simultaneous testing of 8 glucose sensors and successful blood sampling over 14 hours without any complications. Thus a 75% reduction of animals needed can be expected in this kind of studies.

References

Smith, A. C. and Swindle, M. M. (2008). Anesthesia and analgesia in swine. In Fish, R., Danneman, P. J., Brown, M. and Karas, A. (eds.), *Anesthesia and Analgesia in Laboratory Animals* (414-415). Chapter 15, 2nd edition. Academic Press.

Wolfensohn, S. and Lioyd, M. (2006). *Handbook of Laboratory Animal Managment and Welfare* (170-171). Chapter 9, 3rd edition. Blackwell Publishing.

VIII-2-401

MiMyc, a new adjuvant without adverse effects, to replace complete Freund's adjuvant in experimental animal models for human autoimmune diseases

Jennifer Veth, Ella Zuiderwijk-Sick, Linda van Straalen and Jeffrey Bajramovic

Alternatives, Biomedical Primate Research Centre, Rijswijk, The Netherlands
veth@bprc.nl

Adjuvants are formulations that are administered to stimulate immune responses. Besides their use for vaccination purposes, some of the most potent adjuvants like complete Freund's adjuvant (CFA) are used to induce immune responses against components of the body itself in experimental animal models for human auto-immune diseases. CFA is however also notorious for its adverse effects. Most notable is the development of granulomatous skin lesions, causing discomfort to e.g. non-human primates (NHP) in biomedical experiments. There is therefore an urgent need for new and safer adjuvants.

With the recent discovery of families of molecules that form part of the innate immune system, the so called Toll-like and NOD-like receptors (TLR and NLR resp.), important progress has been made in our understanding of how adjuvants work on a molecular level. Our lab has engineered a library of cell lines to become luminescent once different TLR or NLR are engaged. We have used this library to qualitatively and quantitatively profile the innate immune responses that are induced by CFA. Based on these data we have formulated a new synthetic adjuvant to mimic these responses: MiMyc. We will present data comparing adverse effects and adjuvanticity induced by CFA and MiMyc. Our *in vivo* data in NHP demonstrate that MiMyc is a potent adjuvant yet lacks adverse effects. It is therefore a promising candidate to replace CFA. Implementation of MiMyc represents a major Refinement for experiments using NHP, but also for the many other animal species that receive CFA in biomedical research.

VIII-2-169

Ultrasound and infrared thermography as a non-invasive technique to investigate mastitis in sows

Florian Spiegel[1], Sebastian Spiegel[1], Alexandra von Altrock[1], Matthias Lüpke[2] and Michael Wendt[1]

[1]Clinic for Swine and Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [2]Institute for General Radiology and Medical Physics, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany
Spiegel.f@gmx.de

Udder diseases in sows are of large economic importance characterized by reduced milk and high mortality of the piglets.
The purpose of this study was to compare the clinical inspection and palpation of the udder with results of the examination by ultrasound and thermography.

Examination was performed in 107 sows. Thermal images were taken from both sides of the sow picturing all mammary glands. Ultrasound scans were done cranial, caudal and at both sides of the teats of each mammary complex.

Results show that chronic mastitis, which was not detected by clinical diagnostics, could be detected by ultrasound control. Using thermography, these alterations could be illustrated by measuring a decrease in the average surface temperature of the affected area in comparison to the whole complex. The results contribute to improve the diagnostic of udder alterations of sows by using non-invasive imaging techniques as an additional diagnostic tool to classical examination (Refinement).

Session VIII-3: Establishing a Culture of Care Through Assessment, Transparency, and Communication

Co-Chairs
Gregory Reinhard, University of Pennsylvania, Philadelphia, PA, United States
Yumiko Kirihara, Shimane University, Matsue, Japan

VIII-3-725

Culture of care – Thoughts and processes to enhance it

Gregory Reinhard
University of Pennsylvania, Philadelphia, PA, United States
reingr@upenn.edu

An institution with a positive culture of care for laboratory animals is common goal. This presentation will examine some philosophies, methodologies, and practical approaches strengthen scientists' and animal care staff's caring culture for laboratory animals.

VIII-3-19

A culture of care: Animals, people and communication

Bella Williams
Understanding Animal Research, London, United Kingdom
awilliams@uar.org.uk

Public acceptability of animal research, reflected in current legislation, is conditional on both the scientific justification, and the care that is taken of the animals. While facilities place much-warranted emphasis on providing animal care and welfare through the 3Rs, the recent idea of a "culture of care" implies integrated practices that ensure both care of the animals and care of facility staff.

Good science depends on good animal welfare practices, but if staff are to care for their animals effectively then a working environment that values and fosters caring practices is also vital. To help develop more formalized practices around care, UAR has worked with technicians, researchers and stakeholders, mapping their experiences map to existing social and ethical frameworks of caring and care-work.
A clearer understanding of care as an organizational value and working practice, will allow institutions and individuals to take steps that improve both staff and animal welfare. The framework presented here supports strategic steps towards recognizing and building caring practices within laboratory animal facilities.

VIII-3-252

A practical example of measuring culture of care

Thomas Bertelsen
Novo Nordisk A/S, Maaloev, Denmark
tsbt@novonordisk.com

Novo Nordisk has developed a tool to measure the company's culture of care using surrogate markers. Surrogate markers are components that relates to culture of care – the way we behave and the way we think in relation to our work with laboratory animals. The surrogate markers are: three main top-level themes that each has a value-based characteristic – collaboration, trust and integrity, and six operational topics – influence on job situation, meaning of the job, predictability in particular situations, social support, rewards or recognition related to the job and resources to do the job. The survey looks at four different levels: the individual employee, the single groups working with the animals, the management's role and also organisational structures.

The measuring tool is a quantitative survey and it assesses the state of the company's culture of care and it identifies potential gaps. The gap analysis is followed up by qualitative interviews which are essential to initiate action plans in order to assure optimal animal welfare in terms of Reduction and Refinement.

The preliminary pilot study is presented and an outline of potential actions is described.

Reference
Directive 2010/63/EU

VIII-3-685

Delivering a good culture of care

David B. Anderson and Susanna Louhimies
DG Environment, European Commission, Brussels, Belgium
davidbanderson911@gmail.com

A multitude of factors require careful consideration if an effective culture of care is to be delivered. The challenges differ dependent on the nature of the establishment. In all circumstances, there needs to be commitment and support from senior management to ensure appropriate resource and personnel are available.

The structure and processes in place also need to be kept under continuous review to ensure the establishments remain abreast of new innovations in animal welfare, care and use and to ensure effectiveness.

The presentation will explore the various challenges in delivering an effective culture of care, consider how these may be overcome and the opportunities for the different roles to actively contribute to it. Finally, a whole institute-embracing culture of care provides the right environment for a constructive culture of challenge to be established, benefiting both the science and the animals.

Session VIII-4: Ensuring Good Welfare for Genetically Engineered Animals

Co-Chairs
Boris Jerchow, Working Group of Berlin Animal Welfare Officers and Medical Center Hamburg-Eppendorf (UKE), Germany
David Anderson, DG Environment, European Commission, Brussels, Belgium

VIII-4-546

Application of the three Rs in creation and breeding of GA animals

David Anderson and Susanna Louhimies
DG Environment, European Commission, Brussels, Belgium
davidbanderson911@gmail.com

The use of Genetically Altered (GA) animals in scientific procedures continues to increase year on year, indeed in some countries the use of GA mice exceeds the use of conventional animals.

To ensure that the three Rs are effectively implemented, consideration has to be given at all stages of production and maintenance to ensure that the most effective processes and monitoring systems are in place to minimise numbers and degree of suffering.

The presentation will explore the frameworks necessary within establishments to ensure compliance with the Three Rs, how a consistency of approach can be encouraged and how common standards and practices can be encouraged nationally and internationally.

A consistent approach is also necessary to promote a common understanding of the impact of GA manipulation when reporting the severity of scientific procedures.

VIII-4-644

Respecting the 3Rs when using CRISPR technology to generate GE mice: Strategies and comments

Jan Parker-Thornburg
Genetics, MD Anderson Cancer Center, Houston, TX, United States
jpthorn@mdanderson.org

New technologies for genetic engineering may have unintended effects upon the 3Rs requiring consideration. This is particularly relevant with CRISPR technology. For individual projects, CRISPR allows investigators to generate animals easily and cheaply. Thus, one can reduce animal numbers used for an individual project. However, because of the ease of the procedure, many more projects may be attempted, offsetting this reduction. As well, the components of the CRISPR system can be introduced into mice using methods other than standard pronuclear injection, thus providing reduction in animal numbers used and refinements in techniques that benefit the animal. Other reductions in animal use may be obtained by carefully planning genotyping strategies and choice of guide RNAs. Finally, assessment of CRISPR mutations in differentiated ES cells and the use of human cells may partially replace the use of whole animal models, or reduce the numbers of animals required to obtain the correct model.

VIII-4-418

A systematic review of the evidence for discomfort due to toe clipping and ear clipping in laboratory rodents

Marlies Leenaars[1], Kimberley Wever[1], Florentine Geessink[1], Michelle Brouwer[1], Alice Tillema[2] and Merel Ritskes-Hoitinga[1]
[1]SYRCLE Radboudumc, Nijmegen, The Netherlands;
[2]Medical Library, Radboudumc, Nijmegen, The Netherlands
marlies.leenaars@radboudumc.nl

Toe clipping and ear clipping are frequently used for the individual identification of laboratory rodents. These procedures potentially cause severe discomfort, which can reduce animal welfare and distort experimental results. Since no systematic summary of the evidence for discomfort due to toe or ear clipping in rodents currently exists, we performed a systematic review on this topic. We identified 7 studies on the effect of ear clipping on welfare-related outcomes, and 5 such studies on toe clipping. Study characteristics and outcome measures were highly heterogeneous, and there was an unclear or high risk of bias in all studies. Out of > 60 different outcomes, 3 indicated an effect of ear clipping and 4 an effect of toe clipping. In conclusion, the existing body of evidence is too small and of insufficient (reporting) quality to reliably assess the effects of toe or ear clipping. Adequately powered, high-quality studies reporting reliable, relevant outcomes are urgently needed.

VIII-4-367

Ultrasound confirmation of pregnancy in genetically modified mice reduces resource use while enhancing reliability

Michael-Anne Sowick, Jerome Anunciacion, Liza Magee, Mandy Thayer, Jose Diaz, Melanie Domeyer, Alfred Wong, Vida Asghari and Rhonda Wiler

Transgenic Technology, Genentech, Inc., South San Francisco, CA, United States
michaeso@gene.com

We have implemented the use of ultrasound as a non-invasive, early and reliable means to confirm pregnancy in mice.

The mouse is widely used as a model to study embryonic development. The traditional way for assessment of pregnancy in mice is direct visual observation or abdominal palpation, though the reliability of these methods prior to E12.5 depends on the skill of the technician and is dependent on litter size. We have determined that only 60% of females that show evidence of mating are found to be pregnant at the time of ultrasound, allowing us to reuse the non-pregnant animals for other purposes. The ultrasound process involves anesthetizing animals with isoflurane, chemical removal of the abdominal fur, imaging the animals on a heated stage, and monitoring for recovery from anesthesia. The use of this method reduces the impact to our collaborators' experimental timelines and conserves the complex mutant mice.

VIII-4-705

Implement of the 3R principles in genetic modified mice production

Chuan-Kai Chou, Hsian-Jean Chin and Chi-Kuang Wang

National Laboratory Animal Center, NARLabs, Taipei, Taiwan
cck@nlac.narl.org.tw

Genetic modified mice (GM mice) are valuable models for biomedical research, especially in the field of investigating gene function, developmental biology, and diseases. The process of producing GM mice involved several main procedures, often includes superovulation, microinjection, embryo transfer and germline test. Both the production process and the Induced mutation might have significant impact to the welfare of animals. In order to elevate the welfare status in GM mice production, we implement the 3R principles into each procedure. In this report, we demonstrate after optimizing euthanasia methods, embryo transfer settings, animal identification and pain evaluation methods, we not only significantly decreased the number of animal used for GM mice production, and are able to recognize and minimize potential health problems due to gene modification.

VIII-4-365

Welfare assessment and severity classification of genetically altered rodents

Boris Jerchow[1,2], Anne Zintzsch[1,3], Elena Noe[1,4], Monika Reißmann[1,5], Kristina Ullmann[1,6], Stephanie Krämer[1,7], Reinhart Kluge[1,7], Claudia Gösele[1,3], Hannah Nickles[1,6], Astrid Puppe[1,4] and Thomas Rülicke[8]

[1]Working Group of Berlin Animal Welfare Officers, Berlin, Germany; [2]Medical Center Hamburg-Eppendorf (UKE), Research Animal Facilities, Hamburg, Germany; [3]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany; [4]German Rheumatism Research Center (DRFZ), Berlin, Germany; [5]Humboldt University of Berlin, Faculty of Life Sciences, Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Berlin, Germany; [6]Charité – Universitätsmedizin Berlin, Research Institutes for Experimental Medicine (FEM), Berlin, Germany; [7]German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany; [8]Institute of Laboratory Animal Science, University of Veterinary Medicine, Vienna, Austria
b.jerchow@uke.de

Genetically altered (GA) animals are frequently used research models with continuously increasing numbers. Apart from its scientific value, a genetic alteration can compromise an animal's wellbeing. However, the large variety of phenotypes is challenging when it comes to welfare assessment and severity classification, which plays an essential role in pro- and retrospective severity assessment

In Germany, national guidance has been developed on a basic welfare assessment and documentation of strain characteristics. Recently, the Working Group of Berlin Animal Welfare Officers devised an example driven guideline on how to classify different phenotypes into severity categories. The *Guidelines on severity assessment and classification of genetically altered rodents* contain examples of symptoms and syndromes caused by genetic alterations. Examples are assigned to a particular severity category (none, mild, moderate, severe) including recommendations for monitoring and refinement strategies. Beyond the borders of the European Union, this guideline will contribute to the harmonization of severity assessment of genetically altered mice and rat lines.

The presentation gives an overview about the idea of welfare assessment of GA rodents and demonstrates the approach of severity classification on the basis of examples.

Session VIII-5: Humane Endpoints and Euthanasia Considerations for Research Animals

Co-Chairs
Patricia Turner, University of Guelph, Ontario, Canada
Huw Golledge, Universities Federation for Animal Welfare, Wheathampstead, United Kingdom

VIII-5-541

Novel cage-side assessments of post-operative pain in mice

Jennifer Lofgren and Vanessa Oliver
Refinement and Enrichment Advancements Laboratory (REAL), University of Michigan Medical School, Ann Arbor, MI, United States
jlofgren@umich.edu

The ability to rapidly and accurately identify pain in mice is critical for providing them optimal care and welfare. To meet this need, we developed and validated the Grooming Transfer Test (GTT) and Nest Consolidation Test (NCT). We assessed these novel tests along with electronic von Frey and ambulatory parameters at baseline, after isoflurane anesthesia +/- analgesia, and after laparotomy in adult CD1 and C57BL/6 mice of both sexes, housed singly with or without an existing nest or in pairs. While ambulatory parameters had no and von Frey responses minimal significant changes after surgery, GTT and NCT were significantly altered for 48 hours after surgery in both sexes, strains, and across the various housing conditions. Buprenorphine and carprofen each reduced post-operative pain, however only the combination of the two completely prevented delays in nesting behavior. Therefore, these two novel cage-side methods can be used to quickly and objectively identify mice from a variety of signalments and housing conditions with alleviated and unalleviated postoperative pain.

VIII-5-590

Can we diagnose poor welfare at the cage-side? The need to validate welfare tests for diagnostic sensitivity and specificity

Huw Golledge
Universities Federation for Animal Welfare, Wheathampstead, United Kingdom
golledge@ufaw.org.uk

Several techniques have been proposed as diagnostic tests of animal welfare (grimace scales, nesting and burrowing activity etc.), such tools should allow rapid, non-invasive and reliable identification of poor welfare so suffering animals can be treated (or humanely killed). Thus, these tests are analogous to clinical diagnostic tests and must be sensitive (reliably detect poor welfare) and specific (detect animals with good welfare). Some proposed tests do not fulfil these criteria.

Tests unsuitable for cage-side use may have other uses (assessment of retrospective severity or the efficacy of interventions at group level etc.), but can pose risks if relied upon to diagnose poor welfare in individuals. If suffering animals are not detected due to low sensitivity (false negatives) they may go untreated, or be unnecessarily removed from studies and killed. False positives (low specificity) may lead to unnecessary treatment or euthanasia.

I argue there is a need for validation of potential cage-side welfare tests (including systematic review and meta-analysis) to establish their sensitivity, specificity and practicality before they are routinely used.

VIII-5-394

Preventing, recognizing and combating pain in laboratory animals

Marjolein Schilders-van Boxel, Coenraad Hendriksen, Herman Koëter, Jan-Bas Prins, Henriëtte Bout, Wim de Leeuw, Pieter Roelfsema, Frauke Ohl †, Frank Dales and Monique Paris

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands
Marjolein.vanboxel@rvo.nl

In March 2015 the Minister for Agriculture (EZ) commissioned the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) to provide its opinion on the procedure and application of best practices to assist researchers and Animal Welfare Bodies (IvDs) in recognising and managing laboratory animal pain in the workplace, depending on animal species and nature of the animal procedures. At the heart of the advisory report from the NCad is the Code of Practice (CoP) "Prevention, recognition, and management of pain in laboratory animals". This CoP was drawn up by a working group of experts and provides guidance to all parties involved in animal procedures in the prevention, recognition, and management of pain in laboratory animals.

Compelling arguments may be raised for not applying pain management. In response to these, NCad advises that the Central Authority for Scientific Procedures on Animals (CCD): 1) require researchers to provide properly supported arguments justifying the decision to ignore the issue of pain management; 2) focus attention on the non-pharmalogical management of pain, for example by improving husbandry or the application of humane endpoints; 3) keep a register of arguments for not applying pain management, those arguments that are deemed valid, and the results of any additional studies commissioned by the CCD.

Furthermore, the advisory report includes the following recommendations to the Minister: 1) to commission a report into the objective assessment, standardisation, and validation of a pain-scoring system; 2) to make the relevant curriculum committees responsible for ensuring that existing training courses include sufficient focus on pain recognition and management, that the provision of continuous education with regard to pain recognition and pain management is where necessary updated in line with, and responds to, the identified need for visual learning material and e-learning modules; 3) to promote the creation of a network of experts with a (inter)national centralised point of contact. In addition, NCad has taken the lead to bring about cooperation with other EU Member States with the ultimate goal of creating a European knowledge network; 4) The optimal application of the CoP requires supportive measures. Ensure there is an available budget in the years ahead to achieve the goal of limiting to the minimum all forms of pain in animal procedures, as advised in this report.

The minister has accepted and embraced the advisory report and has promoted the CoP as a part of the "Culture of Care" within establishment licensees. Furthermore, in the context of the desire for harmonisation between EU Member States this CoP has been introduced via NCad in European consultations to be used in a wider internationally-supported code of practice Prevention, recognition, and management of pain in laboratory animals.

Reference

https://english.ncadierproevenbeleid.nl/advice/documents/publications/16/7/19/pain-management

VIII-5-23

Severity classification of repeated isoflurane anesthesia in C57BL/6J mice: Assessing well-being and distress

Katharina Hohlbaum[1], Bettina Bert[2,3], Silke Dietze[2], Heidrun Fink[2] and Christa Thöne-Reineke[1]

[1]Institute of Animal Welfare, Animal Behavior and Laboratory Animal Science, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; [2]Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; [3]Current address: Federal Institute for Risk Assessment (BfR), Berlin, Germany
katharina.hohlbaum@fu-berlin.de

According to the Directive 2010/63/EU, the severity of anesthesia is thought to be mild, but the directive does not differentiate between single and repeated anesthesia. Therefore, we investigated the effect of repeated isoflurane anesthesia (6×45 min every 2-3 days) on the well-being and distress of C57BL/6J mice. In addition to the analysis of corticosterone in hair and feces, a behavioral test battery was performed after the last anesthesia. Our results revealed short-term mild distress and impairment of well-being in the early postanesthetic period after both single and repeated anesthesia, although mice recovered more quickly from single than repeated anesthesia. In conclusion, the severity of both single and repeated isoflurane anesthesia in C57BL/6J mice can be classified as mild when adhering to our anesthesia protocol. However, within the mild severity category, repeated anesthesia ranks higher than single anesthesia, with female mice being more vulnerable than male mice.

VIII-5-696

Defining lifetime use and cumulative endpoints for research animals

Patricia Turner, Shawn Petrik and Elizabeth Nunamaker

University of Guelph, Guelph, ON, Canada
pvturner@uoguelph.ca

Humane endpoints for animal studies are refinements and are considered to be the earliest time at which an experimental animal's pain or distress can be avoided or ended by taking actions such as providing euthanasia, relieving pain or terminating the study. Along similar lines, cumulative endpoints may be considered for animals used in more than one protocol for an extended period of time (i.e., lifetime use) or in individual protocols that involve multiple procedures conducted over an extended period of time. We surveyed individuals working in research settings around the world regarding whether and how lifetime use and cumulative endpoints are being tracked and evaluated by Animal Ethics Committees at different institutions, and for which species. Over 150 responses were received, the majority coming from academia, industry, and government. While most Animal Ethics Committees have established formal endpoint policies for experimental use that cover many (although not necessarily all) research species, almost no facility has developed endpoint policies related to frequency of animal use or cumulative lifetime use. The implications will be discussed.

VIII-5-411

Studies on the anesthetic effects of a mixture of medetomidine, midazolam and butorphanol, and antagonism by atipamezole in small rodents

Yumiko Kirihara

Department of Experimental Animals, Shimane University, Izumo, Japan
ukirihar@med.shimane-u.ac.jp

A mixture of medetomidine (MED), midazoram, and butorphanol has been recently used for as an injectable anesthetic in mice and rats instead of the more common ketamine using in Japan. This mixture produced a sufficient anesthetic duration of about 40 minutes in ICR, BALB/c, and C57BL/6 J mice strains by intraperitoneal (IP) injection (Kawai et al., 2011; Kirihara et al., 2013). We also assessed the anesthetic effects of the mixture administered by subcutaneous (SC) and intravenous (IV) injection compared to IP administration. We found that SC injection of the mixture worked equally as well as the IP injection (Kirihara et al., 2015). We compared the effects of the mixture using three different rat strains. We then found that the mixture produced almost the same effects in the rat strains (Kirihara et al., 2016). Atipamezole (ATI) can antagonize an effect of MED. After administration of the mixture, an injection of ATI made the mice and rats rapidly recover from anesthesia (Kirihara et al., 2015, 2016). During the experiment, we measured vital signs using a pulse oximeter. The results may indicate that the anesthetic mixture is an effective anesthesia for laboratory mice and rats. Also, this study may contribute to the welfare of laboratory animals.

References

Kawai, S., Takagi, Y., Kaneko, S. and Kurosawa, T. (2011). Effect of three types of mixed anesthetic agents alternate to ketamine in mice. *Exp Anim 60*, 481-487.

Kirihara, Y., Takechi, M., Kurosaki, K. et al. (2013). Anesthetic effects of a mixture of medetomidine, midazolam and butorphanol in two strains of mice. *Exp Anim 62*, 173-180.

Kirihara, Y., Takechi, M. and Kurosaki, K. (2015). Anesthetic effects of a three-drugs mixture – comparison of administrative routes and antagonistic effects of atipamezole in mice. *Exp Anim 64*, 39-47.

Kirihara, Y., Takechi, M., Kurosaki, K. et al. (2016). Effects of an anesthetic mixture of medetomidine, midazoram, and butorphanol in rats – strain difference and antagonizum by atipamezole. *Exp Anim 65*, 27-36.

Session VIII-6: Training Animals for Better Science and as Partners in the Scientific Process

Co-Chairs
Mary Ann Vasbinder, GlaxoSmithKline, King of Prussia, PA, United States
Lee Niel, University of Guelph, Guelph, ON, Canada

VIII-6-577

Positive reinforcement training for research primates to improve animal welfare and research quality

Mollie Bloomsmith

Animal Resources, Yerkes National Primate Research Center, Atlanta, GA, United States
mabloom@emory.edu

There has been a revolution in care for nonhuman primates in research and testing facilities, and the widespread application of positive reinforcement training methods has been part of this change. Positive reinforcement training is a refinement in animal handling methods that can improve animal welfare, animal husbandry, veterinary care, and research quality. Using positive reinforcement training methods primates are taught to voluntarily cooperate with procedures rather than relying on coercion. They can be taught to cooperate with a variety of procedures that are a routine part of life for research primates including moving between enclosures, allowing examination of parts of their bodies, cooperating with the collection of biological samples (e.g., urine, vaginal fluid, blood) or with receiving injections, and calmly tolerating restraint. Positive reinforcement training can also be used to reduce aggression, fear and abnormal behavior in some cases.

VIII-6-653

Improving cat welfare through better handling

Lee Niel, Carly Moody, Nicole Furgala and Victoria Picketts

Population Medicine, University of Guelph, Guelph, ON, Canada
niell@uoguelph.ca

Many cats display fear and aggression during handling, which can negatively affect welfare, and lead to inadequate physical exams and test results. In response to these concerns, various veterinary organizations are recommending changes to improve cat handling, including managing the environment to minimize potential stressors, and adapting handling by minimizing restraint through alternatives. However, the majority of these recommendations have yet to be assessed scientifically. Our current research is aimed at objectively assessing and improving handling methods for cats to reduce stress during exams and procedures. We will present recent results from a series of studies validating indicators of handling-related stress in cats, and assessing the effects of different environmental changes and handling techniques on cat responses. While the main aim is to improve cat welfare, these results also have the potential to improve research results by reducing stress-related variability.

VIII-6-724

A lifetime of training for dogs in a research environment

Mary Ann Vasbinder[1] and LaVonne Meunier[2]

[1]Office of Animal Welfare, Ethics and Strategy, GlaxoSmithKline, King of Prussia, PA, United States; [2]Global Laboratory Animal Medicine, GlaxoSmithKline, King of Prussia, PA, United States

maryann.8.vasbinder@gsk.com

A quality program for acclimation and socialization of dogs used for research, enhances the welfare of the dogs and staff, facilitates the work and improves scientific outcomes. This is accomplished through effective training programs and acclimation to the study, staff and facility. Optimization of restraint and the study environment improves the animal experience and well-being. Provision of appropriately configured caging and exercise spaces will allow for normal dog behaviors and social interaction. This will allow a smooth transition in the life span of the dog from puppy to the potential opportunity for adoption to a new home (rehoming).

VIII-6-395

Rehoming of former laboratory animals

Marjolein Schilders-van Boxel, Henriëtte Bout, Frauke Ohl †, Herman Koëter, Jan-Bas Prins, Coenraad Hendriksen, Pieter Roelfsema, Wim de Leeuw and Leane van Weereld

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands

Marjolein.vanboxel@rvo.nl

The aim of the advisory report "Rehoming of former laboratory animals" by the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) is to guarantee the quality of life of non-human primates (NHPs), dogs and cats that remain alive at the end of an animal procedure. The terms "putting up for adoption"' and "retiring" are often used for such situations. In its advisory report, the NCad uses the term "rehoming", by which is meant that, remaining alive at the end of an animal procedure, an animal is able to spend the rest of its life at a location suitable for its needs without being subjected to any further animal procedure. Based on the viewpoint that these animals have intrinsic value and should therefore always be treated as sentient beings, one should assume that all dogs, cats and NHPs kept alive (the "yes, unless" principle) are being rehomed.

Various options exist in the Netherlands for rehoming a former laboratory animal. Establishments offering these opportunities develop their own guidelines and procedures. To establish a coordinated and transparent rehoming process, NCad has drawn up a framework that provides a general description of the rehoming process applicable to several different types of animals. In addition, species-specific Codes of Practice have been established for dogs, cats and NHPs.

To encourage the rehoming of eligible cats, dogs and NHPs and to create a coordinated rehoming procedure, the NCad set up recommends with regards to: 1) the encouragement of the adoption of the CoP on cats, dogs and NHPs in practice; 2) the encouragement of a change of attitude within the field, whereby at the end of an experiment animals do not need to be euthanized and can in principle be rehomed, beginning with dogs, cats and NHPs; and 3) the creation of an environment where various parties endeavour to facilitate rehoming, including a balanced division of the attendant costs.

There are also situations in which, for good reasons, rehoming is not an option. These include: 1) The experiment requires the animals are killed because, for example, an autopsy provides essential information; 2) Reuse is possible, compatible with legal guidelines, and ethically acceptable, with consideration having been given to cumulative distress; 3) Laws and regulations prohibit rehoming, for reasons such as risk to public health; or 4) The Animal Welfare Body (IvD) and designated veterinary physician have good reason to believe that the quality of life and life expectancy of the animal will be too low following rehoming.

This poster will give an overview of the advisory report and CoP by the NCad.

Reference

https://english.ncadierproevenbeleid.nl/advice/documents/publications/16/7/19/adoption-of-former-laboratory-animals

VIII-6-737

A personalized medicine approach to behavioral management

Eric Hutchinson

Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD, United States

ehutchi8@jhmi.edu

As environmental enrichment programs have grown in both complexity and ubiquity, the research supporting enrichment decisions has largely focused on identifying best practices to be applied at a species- or strain-level. E.g. mice should be provided nesting material and macaques should have fully access to appropriate social partners. Applying a uniform standard to as large a population as possible has been mostly successful, and indeed makes enrichment in large institutions possible. However, less attention has been paid to determining which subgroups or individual animals may benefit from behavioral management strategies other than the "standard." This presentation will raise examples from the literature of populations that appear not to benefit from the "one size fits all" approach, and how approaching behavioral management from a personalized medicine perspective can help improve the welfare or these animals.

References

Coleman, K., Tully, L. A., McMillan, J. L. (2005). Temperament correlates with training success in adult rhesus macaques. *Am J Primatol 65*, 63-71.

Hutchinson, E. K., Avery, A. C. and Vandewoude, S. (2012). Environmental enrichment during rearing alters corticosterone levels, thymocyte numbers, and aggression in female BALB/c mice. *J Am Assoc Lab Anim Sci 51*, 18-24.

Meade, T. M., Hutchinson, E., Krall, C. and Watson, J. (2014). Use of an aquarium as a novel enrichment item for singly housed rhesus macaques (Macaca mulatta). *J Am Assoc Lab Anim Sci 53*, 472-477.

Session VIII-7: Promotion of the 3Rs and Research Reproducibility

Co-Chairs
Paul Honess, Independent Consultant, Oxford, United Kingdom
Norman Peterson, Medimmune, Gaithersburg, MD, United States

VIII-7-594

More than 3Rs – The 3Vs to improve the validity and reproducibility of animal research

Hanno Wuerbel
Division of Animal Welfare, University of Bern, Bern, Switzerland
hanno.wuerbel@vetsuisse.unibe.ch

The 3Rs concept serves to minimize harm in research animals. Whether the use of animals is justifiable, however, depends also on the expected benefit of the research. Unless study outcomes are valid and reproducible, animals may be wasted for inconclusive research. Accumulating evidence indicates risks of bias caused by flaws in the design and conduct of animal research. I therefore propose a more systematic assessment of scientific validity when reviewing grant proposals, study protocols, and publication manuscripts, including evidence of construct validity, internal validity, and external validity of the expected outcomes. As with the 3Rs, there is no need for a fixed checklist approach. Instead, criteria for assessing the 3Vs could be defined according to the decisions to be taken (e.g. project funding, protocol approval, publication). Together with the 3Rs, the 3Vs would thus help to avoid wasting animals for inconclusive research and imposing unnecessary harm on research animals.

VIII-7-588

An omics based approach to improving reproducibility in animal-based studies

Norman Peterson
Translational Sciences, Medimmune, Gaithersburg, MD, United States
jtnp@comcast.net

The reproducibility of *in vivo* research has been attributed to a lack of multiple factors including: environment, genetics, microbiome, technique, bias, statistics, and adequate reporting. Controlling all of these variables is not feasible or practical. By adapting an omics-based approach, reproducibility in animal studies could be greatly improved. In these omics studies, standard housekeeping biomarkers are measured for quality assurance and as comparators with each data set. In adapting this strategy to *in vivo* studies, these biomarkers could be physiologic, metabolic, cellular, or molecular in nature (or any combination thereof), and ideally sampled prior to initiation (baseline data) and longitudinally. Examples will be provided. By adapting this strategy to *in vivo* studies, greater context of the system's microenvironment would be provided, interpretation of results would be facilitated, and outcomes could be more readily standardized across different laboratories.

VIII-7-5

PREPARE guidelines for planning animal research and testing

Adrian Smith[1], Eddie Clutton[2], Elliot Lilley[3], Kristine Hansen[4] and Trond Brattelid[5]
[1]Norecopa, Oslo, Norway; [2]Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom; [3]RSPCA, Southwater, United Kingdom; [4]Norwegian University of Life Sciences, Oslo, Norway; [5]Western Norway University of Applied Sciences, Bergen, Norway
adrian.smith@norecopa.no

In attempts to improve the validity, reproducibility and translatability of animal experiments, a number of reporting guidelines have been written. There are, however, many other factors, seldom reported in the scientific literature, which can influence the outcome of experiments, animal welfare, and the health and safety of all concerned.

We have produced guidelines for planning animal experiments, called PREPARE (*Planning Research involving Experimental Procedures on Animals: Recommendations for Excellence*). PREPARE covers all stages of quality assurance, from the management of an animal facility to individual procedures. They are also relevant to field experiments. More information is available on the PREPARE website: https://norecopa.no/PREPARE.

VIII-7-667

Global enrichment challenges for non-human primates: From the lowest to the highest hanging fruit

Paul Honess[1] and Paul Finnemore[2]
[1]Independent Consultant, Oxford, United Kingdom; [2]AstraZeneca/MedImmune, Cambridge, United Kingdom
pehoness@gmail.com

All primate facilities face challenges in their enrichment strategies. Where there is little historical experience of fine-tuned enrichment plans, animal housing can tend towards the basic. This leaves small, typically inexpensive, enrichments (often widespread in Europe and N. America) available to improve welfare substantially. In more mature contexts, the lower hanging fruit have often already been picked. Here challenges persist that can be hard to meet, including keeping enrichment strategies appropriate and novel in the face of evolving regulations and best practice recommendations. To improve global standards we must encourage those yet to pick the low-hanging fruit to do so successfully and safely, rather than requiring all fruit to be picked at once. This pragmatic approach builds a sustainable enrichment culture that recognises the positive impact of effective enrichment, while addressing the reproducibility challenges resulting from globally varying enrichment standards.

VIII-7-692

Gut microbiota and animal model reproducibility

Patricia Turner
University of Guelph, Guelph, ON, Canada
pvturner@uoguelph.ca

The gut microbiota is composed of up to 10^{14} cells and their various metabolites living, dying, and reproducing throughout the gastrointestinal tract of mammals. It co-evolved with each host species to assist with day to day functions, contributing to the overall health of animals in remarkable ways. Because of the significant beneficial impact that gut microbiota may have on other organ systems there is interest in learning more about the gut microbiota and translating these findings into clinical therapies. Results from recent studies characterizing the gut microbiota have demonstrated that many factors may affect gut microbiota diversity. Relatively little is known about the functional consequences of alterations of the gut microbiota and exactly how changes in richness and diversity of the microbiota result in changes in health and susceptibility to disease. Questions have also been raised as to whether ultraclean, barrier-raised mice are relevant models of human disease, given their reduced gut microbiota diversity and complexity. This talk will explore animal model reproducibility in light of new findings about the gut microbiota.

VIII-7-205

Characterizing the variability of LD_{50} values in acute toxicity studies: Implications for alternative methods development

AL Karmaus[1], Dave Allen[1], Nicole Kleinstreuer[2] and Warren Casey[2]
[1]ILS, Morrisville, NC, United States; [2]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, United States
agnes.karmaus@nih.gov

In vivo LD_{50} values are often used as reference data to evaluate alternative methods to estimate acute toxicity. However, to achieve a fair assessment of alternative methods, it is important to determine the extent to which *in vivo* studies vary or predict themselves. We obtained LD_{50} values from multiple databases, including the NLM's Hazardous Substances Data Bank and ChemIDplus, the OECD's eChemPortal, and the JRC's AcutoxBase, yielding a total of 27,380 oral LD_{50} values representing 11,276 unique chemicals and 13 species. All chemicals with ≥ 5 studies had variable LD_{50}s spanning at least one order of magnitude, with some ranging over four orders of magnitude, not only across rat studies but also across multiple species. These results underscore the importance of considering an appropriate margin of uncertainty when using *in vivo* acute oral toxicity data for the assessment of alternative methods.

U.S. Federal funds from NIEHS/NIH/HHS contract HHSN273201500010C supported this study.

VIII-7-117

A welfare assessment framework for understanding and improving welfare and data output in the dog

Laura Scullion Hall[1], Sally Robinson[2], Jackie Boxall[3] and Hannah Buchanan-Smith[1]
[1]Psychology, University of Stirling, Stirling, United Kingdom; [2]AstraZeneca, Macclesfield, United Kingdom; [3]GlaxoSmithKline, Ware, United Kingdom
laura.hall@stir.ac.uk

Dogs are the most commonly used non-rodent species in safety assessment of new medicines with > 100,000 used per year. Previous research has identified a need to better measure welfare and the concomitant link with data quality in the laboratory-housed dog (Prescott et al., 2004). Crucial decisions relating to housing, husbandry and regulated procedures may be based on anecdotal rather than empirical evidence and effects of planned Refinements not measured to identify welfare benefits. We developed a Welfare Assessment Framework (Scullion Hall et al., in prep) that describes a system of measurement for welfare and data output including behaviour, affect, clinical pathology, mechanical threshold and cardiovascular output. Data will be presented from dogs (n = 200) housed in different units with contrasting histories of regulated procedures, housing, husbandry and training techniques. The Welfare Assessment Framework has been employed to examine areas in need of Refinement and devise Refinements to benefit welfare and data output, such as improved home pen and facility design (Scullion Hall et al., 2017), training (Scullion Hall and Robinson, 2016), dosing (Hall et al., 2015) and signalled predictability (Scullion Hall et al., submitted).

References

Hall, L. E., Robinson, S. and Buchanan-Smith, H. M. (2015). Refining dosing by oral gavage in the dog: A protocol to harmonise welfare. *J Pharmacol Toxicol Methods 72*, 35-46.

Prescott, M. J., Morton, D. B., Anderson, D. et al. (2004). Refining dog husbandry and care. *Lab Anim 38*, 96.

Scullion Hall, L. E. M. and Robinson, S. (2016). Implementing a successful positive reinforcement training protocol in laboratory-housed dogs. *Animal Technology and Welfare 15*.

Scullion Hall, L. E. M., Robinson, S., Finch, J. and Buchanan-Smith, H. M. (2017). The influence of facility and home pen design on the welfare of the laboratory-housed dog. *J Pharmacol Toxicol Methods 83*, 21-29.

Scullion Hall, L. E. M, Robinson, S., Finch, J. and Buchanan-Smith, H. M. (submitted). Anticipation and predictability as tools to improve welfare in laboratory-housed dogs.

Scullion Hall, L. E. M., Robinson, S. and Buchanan-Smith, H. M. (in prep). A welfare assessment framework for the laboratory-housed dog: Linking welfare and quality of data output.

Statistics, regulations and legislation. Refining Dog Care. http://www.refiningdogcare.com/resources/stats/ (retrieved 24 Apr 2017).

Session VIII-8: Advances in Recognition and Treatment of Pain in Laboratory Rodents

Co-Chairs
Nathalie Percie du Sert, NC3Rs, London, United Kingdom
Marilyn Brown, Charles River Laboratories, Wilmington, MA, United States

VIII-8-747

Pain assessment and new innovations

Sara Wells
Mary Lyon Centre, Medical Research Council, Oxon, United Kingdom
s.wells@har.mrc.ac.uk

For many years animal care workers have been striving to refine the assessment of pain in rodents by scoring behavioural and visible characteristics indicative of discomfort. Being able to focus on particularly relevant times such as post-surgery or after treatment facilitates early intervention and amelioration. However, where the source of the pain is present throughout the lifespan of the animal, but may manifest itself sporadically and unpredictably, it is still very difficult to provide effective management. This is particularly the case with genetically altered (GA) animals carrying debilitating mutations or of newly generated GA strains where the phenotype has not yet been characterised. To overcome the constraints of manual observations including long periods of assessment time, disturbing animals from their usual environment and lighting conditions, it is necessary to develop new ways of assessing pain.

Home cage monitoring equipment is being developed to record laboratory rodents 24 hours a day, in their home cages and throughout dark periods. This will greatly facilitate the detection of different patterns of activity and social behaviours that will serve as early indicators of pain and discomfort, thereby facilitating early intervention and care.

VIII-8-716

Preventing and alleviating pain associated with experimental procedures in laboratory animals

Paul Flecknell
Newcastle University and Flair Consultants, Newcastle, United Kingdom
paul.flecknell@newcastle.ac.uk

The effective alleviation of procedure-related pain in laboratory animals is an important goal. Despite the emphasis given to humane treatment of laboratory animals in the national legislation of many countries, analgesics may still not be administered routinely in the post-operative period. This omission is particularly common in studies using small rodents.

Early misconceptions about the nature of animal pain limited effective pain management in all species, but this problem has now largely been overcome. The risk of clinically significant side-effects of analgesics has also limited their use, but this concern has reduced as our understanding of species differences in the activity of analgesics has increased. A wide range of different analgesic agents are available, and many of these underwent preclinical assessment of efficacy and safety in small rodents. Potentially safe and effective analgesics are therefore available. However, we still lack data on the clinical efficacy and duration of action of many of these agents. This is reflected in the wide range of doses reported in the literature, and the widely varying duration of treatments. The potential role of novel products, such as slow-release formulations and techniques such as epidural and intrathecal routes of administration have also not been properly evaluated. Finally, analgesic use rarely involves multimodal therapy, and structured therapeutic approaches analogous to the WHO Pain Ladder are not used.

It is also necessary to incorporate pain assessment and management into an overall scheme of perioperative care. We need to be concerned with distress, as well as pain, and should be aware that practices such as handling methods can significantly influence animal stress, as can anaesthesia, intraoperative care, and postoperative management. Increased stress can increase pain and reduce the efficacy of analgesics. Attention to all of these factors is necessary if we are to refine research procedures effectively.

VIII-8-586

Effects of analgesics and pain on research outcomes

Norman Peterson
Translational Sciences, Medimmune, Gaithersburg, MD, United States
jtnp@comcast.net

A major challenge faced by scientists and bioethics review committees in evaluating several research proposals is in predetermining how unrelieved pain or the choice of analgesics can influence outcomes. There tends to be a bias toward the negative impact of the latter, and this is likely the result of the perception that the addition of pharmaceuticals adds unknown variables and that efforts needed to treat and monitor pain have little scientific value. Consideration in the assessment of requests for exemption to the use of analgesics will be discussed. Several studies demonstrate that the molecular and physiologic effects of unalleviated pain upon the model need to be considered and that lack of intervention may adversely affect outcomes. Additionally, the translational relevance of the models must be considered when the use of analgesics is questioned. The information provided here will help scientists and reviewers make sound decisions on the use analgesics in *in vivo* studies.

Theme VIII: Refinement and Animal Welfare

Poster Presentations

VIII-12

Strain-dependent metabolism and analgesic efficacy of buprenorphine in mice

Juliane Rudeck[1], Silvia Vogl[1], Bettina Bert[1], Stefanie Banneke[1] and Gilbert Schönfelder[1,2]

[1]German Federal Institute for Risk Assessment, Berlin, Germany; [2]Charité – Universitätsmedizin Berlin, Berlin, Germany
juliane.rudeck@bfr.bund.de

Buprenorphine is a commonly used analgesic for postsurgical pain treatment in mice. Effective pain treatment is challenging due to different aspects e.g. strain, age, sex. The aim of our study is to investigate the influence of strain differences on analgesic efficacy of buprenorphine and on its phase I and II metabolism. We compared male mice, 6 to 8 weeks old, of three different inbred mouse strains: C57BL/6J, Balb/cJ and 129S1/SvImJ. To determine strain differences for the analgesic efficacy, basal pain sensitivity and pain sensitivity were tested for every mouse in the incremental hot plate test 30 minutes after s.c. injection of buprenorphine. After testing, mice were immediately sacrificed with CO_2 to sample blood and to remove brain and liver for further analysis. Preliminary data suggest that strain differences exist for analgesic efficacy of buprenorphine. The results and conclusions of this study can contribute to a refined pain management for different mouse strains.

VIII-31

A 3Rs study: Comparison of blood collection methods for hematology and clinical chemistry from C57BL/6 mice

Alysha M. Hoffman[1], Lindsay Martin[2], Jimmy Tang[2], Nancy E. Everds[2] and Madhu P. Sirivelu[2]

[1]Comparative Animal Research, Amgen Inc., South San Francisco, CA, United States; [2]Comparative Biology and Safety Sciences Lab, Amgen Inc., Seattle, WA, United States
alysha@amgen.com

The principles of the 3R's are a framework for conducting quality, humane research throughout the industry. The objective of this study was to identify best practices for in-life and terminal blood sample collection procedures in an effort to refine our bio methodology techniques to prevent the need for additional invasive collection methods and reduce the potential number of animals impacted by repeat studies. Differences in sample quality among five in-life sampling techniques and three terminal blood collection methods in 10 week old, female C57BL/6 mice were evaluated. A blocking design for sample collection minimized bias in order of collection, timing, and phlebotomy skill. Results of this study identified tail venipuncture method as having the best sample integrity with the parameters measured. This study provides helpful guidelines for obtaining high-quality samples and demonstrates the substantial impact of phlebotomy method on clinical pathology results.

References

Doeing, D. C. et al. (2003). Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clinical Pathology 3*, 1-6.

Nemzek, J. A. et al. (2001). Differences in normal values for murine white blood cell counts and other hematological parameters based on sampling site. *Inflamm Res 50*, 523-527

VIII-48

Refining dog care: Evidence-based refinements to improve dog welfare and data output

Laura Scullion Hall and Hannah Buchanan-Smith

Psychology, University of Stirling, Stirling, United Kingdom
laura.hall@stir.ac.uk

The dog is the most commonly used non-rodent species in the safety assessment of new chemical entities (> 100,000 pa) yet we know little about their welfare and impact of routine practices on welfare (Prescott et al., 2004). While there is broad desire to implement effective Refinements to many aspects of dog use there are barriers to uptake, including lack of evidence and resources specific to the research environment, and concerns about interference with data quality and study outputs. From our collaborative project across UK industry (http://www.refiningdogcare.com), we present evidence-based resources for good practice and a number of protocols will be shared. Techniques to improve welfare and data output, and prepare dogs for study life will be presented. These include facility and home pen design (Scullion Hall et al., 2017), enrichment (Hall, 2014), training (Scullion Hall and Robinson, 2016), predictability (Scullion Hall et al., submitted), handling and dosing techniques (Hall et al., 2015). We describe empirical evidence demonstrating both welfare benefits and ease of implementation of an effective training protocol for laboratory-housed dogs are described. Our welfare assessment framework (Hall et al., 2015) is employed to monitor the impact of planned Refinements on welfare, and to evaluate preparation for procedures.

References

Hall, L. E. (2014). A practical framework for harmonising welfare and quality of data output in the laboratory-housed dog. Unpublished thesis. University of Stirling, Stirling, UK.

Hall, L. E., Robinson, S. and Buchanan-Smith, H. M. (2015). Refining dosing by oral gavage in the dog: A protocol to harmonise welfare. *J Pharmacol Toxicol Meth 72*, 35-46.

Prescott, M. J., Morton, D. B., Anderson, D. et al. (2004). Refining dog husbandry and care. *Lab Anim 38*, 96.

Scullion Hall, L. E. M. and Robinson, S. (2016). Implementing a successful positive reinforcement training protocol in laboratory-housed dogs. *Animal Technology and Welfare 15*.

Scullion Hall, L. E. M., Robinson, S., Finch, J. and Buchanan-Smith, H. M. (2017). The influence of facility and home pen design on the welfare of the laboratory-housed dog. *J Pharmacol Toxicol Methods, 83*, 21-29.

Scullion Hall, L. E. M., Robinson, S., Finch, J. and Buchanan-Smith, H. M. (submitted). Anticipation and predictability as tools to improve welfare in laboratory-housed dogs.

VIII-56

Animal use training program at the University of Washington and the 3Rs

Erika French, Kathy Andrich and Jennifer Linton

Office of Animal Welfare, University of Washington, Seattle, WA, United States
ef7@uw.edu

Replacement: The training program provides tissues from animals euthanized in training classes to researchers. This allows researchers to avoid having to use their own live animals for collecting tissues and gives further purpose to the training animal colony.

Reduction: The training program's mouse and rat colonies are donated from researchers who no longer have a use for the animals (e.g., retired breeders or the incorrect genotype). Reduction is further increased with careful tracking of all animal procedures; animals used for terminal procedures will have already been used for other less invasive procedures.

Refinement: The training program has recently learned of and incorporated a new method of obtaining blood on awake mice called the Submental Bleed (Regan et al., 2016). Teaching this method involves less mortality and morbidity and students catch on quickly. A comparison to other bleeding methods is emphasized.

Reference

Regan, R. D., Fenyk-Melody, J. E., Tran, S. M. et al. (2016). Comparison of Submental Blood Collection with the Retroorbital and Submandibular Methods in Mice (Mus musculus). *J Am Assoc Lab Anim Sci 55*, 570-576.

VIII-65

Study of an alternative *in vitro* pyrogen test for blood products using monocyte activation test

Hoil Kang, Kikyung Jung and Jihye Kim

Blood Products Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, South Korea
kanghoil@korea.kr

The rabbit pyrogen test (RPT) is performed for the national lot release of blood products such as human serum albumin, immunoglobulin, coagulation factor, etc. However, it costs many lives of rabbits and it does not line with the global trend, also represented as 3R. The aim of our study is to develop an alternative method for this animal test.

We have studied on monocyte activation test (MAT) as an alternative method for the RPT. We used rabbit PBMCs as a source of monocyte and employed ELISA to measure the cytokines released from monocyte.

As a result, we established an assay protocol of *in vitro* pyrogen test utilizing monocyte activation test. And we compared the results obtained by RPT and MAT for some important pyrogens to examine the possibility to replace RPT to MAT. The temperature rise in RPT and increase of cytokines released in MAT show a similar trend. The results of this research will be used as baseline data for introduction of *in vitro* pyrogen test and also contribute to improvement of quality control for blood products.

References

Hartung, T., Aaberge, I. and Berthold, S. (2001). Novel pyrogen tests based on the human fever reaction. *Altern Lab Anim 29*, 99-123.

Hoffmann, S., Peterbauer, A., Schindler, S. et al. (2005). International validation of novel pyrogen tests based on human monocytoid cells. *J Immunol Methods 298*, 161-173.

Fennrich, S., Fischer, M., Hartung, T. et al. (1999). Detection of endotoxins and other pyrogens using human whole blood. *Dev Biol Stand 101*, 131-139.

Koryakina, A., Frey, E. and Bruegger, P. (2014). Cryopreservation of human monocytes for pharmacopeial monocyte activation test. *J Immunol Methods 450*, 181-191.

Schindler, S., Spreizer, I. and Loschner, B. (2006). International validation of pyrogen tests based on cyropreserved human primary blood cells. *J Immunol Methods 316*, 42-51.

VIII-74

Impact of stripping technique in skin penetration studies on rate of dermal absorption

Daniela Lange, Ilka Herberz, Julia Mikolon, Mehdi Akhiani, Horst Wenck and Andreas Schepky

Beiersdorf AG, Hamburg, Germany
daniela.lange@beiersdorf.com

A key parameter for risk assessment of topically applied products is the determination of the bioavailability in different skin layers. The discrimination between systemically available substance and the amount remaining in the stratum corneum plays an important role to estimate potential toxicological effects. Therefore, the stratum corneum has to be analyzed separately from the living epidermis after skin penetration studies. We compared the stripping efficacy of different tape strips and the stripping depth when using 16, 30 or 50 strips as well as cyanoacrylate in cryosections. We observed vast differences in the stripping depth depending on the used tape strip and the no. of tape strips. In skin penetration studies we could show that removing the stratum corneum with 16 or 50 strips has an impact on the amount found as dermally absorbed and therefore on the toxicological assessment of the used substance.

VIII-99

Refining for success: Strategies for inducing robust immune responses and implementing exercise pens as enrichment for laboratory rabbits

Kellen Schneider, Raul Garcia-Gonzalez, Zhonghua Lin, WeiYu Lin, Scott Stawicki, Jill Yamada, Emmanuel Chua, Ryan Scott, Katelynn McEachin, Catherine Sohn and Dhaya Seshasayee

Genentech, Redwood City, CA, United States
schnek20@gene.com

Rabbits are commonly used in research to generate antibodies. Antigen injections using adjuvants cause adverse effects. We refined all our methods, such as lower volumes, dosing routes, blood collection, a safer CFA strain and increased post-immunization monitoring. We reduced animal usage to 2 rabbits (vs 10-15 rodents) per antigen, as rabbits generate large, diverse, high-affinity antibody panels. We have built a strong antibody discovery platform, with robust immune responses, while reducing animal usage and health issues. In addition, caged lab rabbits can exhibit abnormal and repetitive behaviors (stereotypes). Rabbits were housed in new European-style cages, with shelves and toys. A pilot exercise program was set up by the veterinary staff: 7 rabbits individually placed into large pens, 5 days/week, 30 minutes daily. Pens were designed to be easy to sanitize. Exercised rabbits displayed more social behaviors. Some were eventually retired from research and successfully adopted.

References

Buijs, S., Keeling, L. J., Rettenbacher, S. et al. (2011). Glucocorticoid metabolites in rabbit faeces – Influence of environmental enrichment and cage size. *Physiol Behav 104*, 469-473.

Dixon, L. M., Hardiman, J. R. and Cooper, J. J. (2010). The effects of spatial restriction on the behavior of rabbits (Oryctolagus cuniculus). *J Vet Behav 5*, 302-308.

Gunn, D. and Morton, D. B. (1995). Inventory of the behaviour of New Zealand White rabbits in laboratory cages. *Appl Anim Behav Sci 45*, 277-292.

Hansen, L. T. and Berthelsen, H. (2000). The effect of environmental enrichment on the behaviour of caged rabbits (Oryctolagus cuniculus). *Appl Anim Behav Sci 68*, 163-178.

Knutsson, M. (2011). Exercise pens as an environmental enrichment for laboratory rabbits. Unpublished thesis, Swedish University of Agricultural Sciences.

Lidfors, L. and Edström, T. (2010). The laboratory rabbit. In R. Hubrecht and J. Kirkwood (eds.), *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*. 8th edition. 399-417. Oxford: Wiley-Blackwell.

Swennes, A. G., Alworth, L. C., Harvey, S. B. et al. (2011). Human handling promotes compliant behavior in adult laboratory rabbits. *J Am Assoc Lab Anim Sci 50*, 41-45.

Trocino, A. and Xiccato, G. (2010). Animal welfare in reared rabbits: A review with emphasis on housing systems. *World Rabbit Science 14*, 77-93.

VIII-185

Development of the LLNA: BrdU-FCM for skin sensitization evaluation

Ilyoung Ahn[1], Jung-Sun Yi[1], Byeol I Han[1], Souk Jin Seo[1], Mi Hye Hong[1], Kyungyuk Ko[1], Ji Young Kim[1], Tae Sung Kim[1], Joohwan Kim[1], Hye-Kyung Park[2] and Jong Kwon Lee[1]

[1]Toxicological Screening & Testing Division, National Institute of Food and Drug Safety Evaluation, South Korea; [2]Toxicological Evaluation and Research Department, National Institute of Food and Drug Safety Evaluation, South Korea

ahnilyoung17@korea.kr

The LLNA: BrdU FCM, which is a new non-radioactive LLNA variant, was developed by the NIFDS and can be employed in assessing T-cell proliferation by measuring BrdU-positive cells with flow-cytometry. The LLNA: BrdU-FCM validation has been performed according to the validation principles in OECD GD 34 and using the reference chemicals listed in Annex 1 of TG 429. Predictive capacity was tested using the 22 reference chemicals. The test results showed 91% accuracy compared with the LLNA. These results met the criteria in GD 34 and the PS. The LLNA: BrdU-FCM provides opportunities to use BALB/c mice in the LLNA method in countries where the mouse strains usually used, such as CBA/J, are not readily available. The assay can also detect additional immune cytokines without sacrificing extra animals. The LLNA: BrdU-FCM is expected to predict skin sensitization and allow for the monitoring of immunotyping parameters relevant for skin sensitisation. This research was supported by a grant (17181MFDS486) from the Ministry of Food and Drug Safety in 2017.

References

Ahn, I. Y., Kim, T. S., Jung, E. S. et al. (2016). Performance standards based validation study for local lymph node assay: 5-Bromo-2-Deoxyuridine-flow cytometry method. *Regul Toxicol Pharmacol 80*, 183-194.

Ha, S., Ahn, I. Y., Kim, D. E. et al. (2017). Evaluation of radioisotopic and non-radioisotopic versions of local lymph node assays for subcategorization of skin sensitizers compliant to UN GHS rev 4. *Regul Toxicol Pharmacol 85*, 124-131.

Lee, Y. S., Yi, J. S., Seo, S. J. et al. (2017). Comparison of BALB/c and CBA/J mice for the local lymph node assay using bromodeoxyuridine with flow cytometry (LLNA: BrdU-FCM). *Regul Toxicol Pharmacol 33*, 13-22.

Yang, H., Na, J., Jang, W. H. et al. (2015). Appraisal of within- and between-laboratory reproducibility of non-radioisotopic local lymph node assay using flow cytometry, LLNA: BrdU-FCM: Comparison of OECD TG429 performance standard and statistical evaluation. *Toxicol Letters 234*, 172-179.

VIII-216

The use of cell culture as an alternative to study the immune response against *Encephalitozoon cuniculi*

Maria Anete Lallo[1], Adriano Pereira[1], Fabiana Toshie de Camargo Konno[1], Elizabeth Christina Perez[1], Diva Denelle Spadacci-Morena[2] and Anuska Marcelino Alvares-Saraiva[1]

[1]Environmental and Experimental Pathology, Paulista University, Recife, PE, Brazil; [2]Butantan Institute, Butantã, SP, Brazil

anetelallo@hotmail.com

Current understanding of the immune response against *Encephalitozoon cuniculi* infection has derived mostly from researches using immunodeficient and immunocompetent mice. To understand the role of some immune mechanisms against *E. cuniculi* and thinking about 3 R's to reduce the number of animals, we decided to study *in vitro* the B-1 cell influence on the phagocytic capacity (PC) of macrophages. Adherent Peritoneal Cells (APerC) from BALB/c mice (with macrophages and B-1 cells) and APerC from XID mice (B-1 cells deficient) were infected with *E. cuniculi*. After 1 h and 48 h were collected to analyze the PC using a light and transmission electron microscopy (TEM). The PC of APerC from XID mice was significantly higher than that of APerC from BALB/c mice. By TEM, we observed a higher number of *E. cuniculi* spores within macrophages from XID, with its preserved integrity. These data suggest that B-1 cells are able to down-regulate the efficacy of macrophages to kill pathogens.

References

da Costa, L. F., Alvares-Saraiva, A M., Dell`Armelina Rocha, P. R. et al. (2017). B-1 cell decreases susceptibility to encephalitozoonosis in mice. *Immunobiology 222*, 218-227.

Haas, K. M., Poe, J. C., Steeber, D. A. and Tedder, T. F. (2005). B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to S. pneumonia. *Immunity 23*, 7-18.

Mussalem, J. S., Squaiella-Baptistão, C. C., Teixeira, D. et al. (2012). Adjuvant effect of killed Propionibacterium acnes on mouse peritoneal B-1 lymphocytes and their early phagocyte differentiation. *PLoS One 7*, e33955.

VIII-308

Further validation of the CULTEX® RFS method and optimization of a prediction model to evaluate the acute toxicity of inhalable dusts

Amelie Tsoutsoulopoulos[1], Niklas Möhle[2], Katrin Gohlsch[3], Andreas Breit[3], Harald Mückter[3], Thomas Gudermann[3], Olaf Krischenowski[2], Sebastian Hoffmann[4], Horst Thiermann[1], Dirk Steinritz[1,3] and Michaela Aufderheide[2]

[1]Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany; [2]Cultex Laboratories GmbH, Hannover, Germany; [3]Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität, Munich, Germany; [4]seh consulting + services, Paderborn, Germany

amelietsoutsoulopoulos@gmail.com

Assessment of acute lung toxicity originating from airborne particles is a regulatory requirement, but information is often lacking. In addition, EU-regulation REACH demands to implement the 3R concept of refinement, reduction and replacement of animal experiments. The CULTEX® Radial Flow System (RFS) exposes human lung cells to clean air or defined particle concentrations at the air-liquid interface to mimic the *in vivo* situation of alveoli exposure. After 24 hours cell viability was assessed using the WST-1 assay. This allows the description of toxicological properties, thereby potentially replacing animal experiments.

In the frame of validation activities, harmonization and refinement of the procedure resulted in highly stable and reproducible results across the three cooperating laboratories. Overall results confirm the findings of our pre-validation study and highlight the method as an innovative 3R-conform approach for analyzing acute toxicity of inhalable substances *in vitro*.

VIII-314

Environmental enrichment in regulatory toxicological studies – skin sensitization test

Przemyslaw Fochtman, Anna Rakowska, Inga Mrzyk, Malgorzata Przybyla, Patrycja Florek and Aneta Kropidlo

Institute of Industrial Organic Chemistry Branch Pszczyna, Pszczyna, Poland

fochtman@ipo-pszczyna.pl

Laboratory studies with vertebrate animals performed due to regulatory requirements to be accepted have to follow specific test guidelines/methods as well as up-to-date law regulations. Testing for skin sensitization in case of plant protection products or chemical substances in European Union has to follow OECD Test Guideline No 406 (adopted July 17, 1992). Guinea pig has been the animal of choice for predictive skin sensitization tests for several decades. Performing tests with vertebrate animals requires also that animal welfare shall not be compromised. European Union Directive 2010/63/EU (adopted September 22, 2010) requires that animals shall be provided with environmental enrichment. We compare results of mercaptobenzothiazole skin sensitization test performed in compliance with conventional OECD Test Guideline No 406 and modified by inclusion of guinea pig environmental enrichments elements.

References

Directive 2010/63/EU on the protection of animals used for scientific purposes. *OJ EU L 276*, 33.

OECD Test Guideline No 406: Skin sensitization (adopted July 17, 1992).

VIII-412

A comparison of former laboratory dogs' with non-laboratory dogs' psychological and behavioral characteristics

Stacy Lopresti-Goodman

Psychology, Marymount University, Arlington, VA, United States

stacy.lopresti-goodman@marymount.edu

It is becoming more common for laboratories to release dogs for adoption when they are no longer needed in experiments (U.C. Davies, 2017). Previous research demonstrates that dogs subjected to chronic stress and inadequate housing conditions in laboratories may develop generalized fearfulness, anxiety, and engage in abnormal behaviors (Beerda et al., 1997, 1999, 2000; Hett et al., 1992). This study used the Canine Behavioral Assessment and Research Questionnaire (Hsu and Serpell, 2003) to compare psychological and behavioral characteristics of 113 dogs released from laboratories to a convenience sample of 418 dogs who had no known history of laboratory use. The results reveal that former laboratory dogs exhibit increased fear of strangers and in non-social situations, and engage in more abnormal behaviors than the convenience sample, however, they have increased attachment to their caregivers, are less aggressive, and do not differ on many behaviors assessed, including trainability. The findings demonstrate that former laboratory dogs can adjust to living in private homes despite their negative early life experiences, and support the ongoing work by laboratories and animal rescue organizations to offer these dogs for adoption.

References

Beerda, B., Schilder, M. B. H., van Hooff, J. and De Vries, H. W. (1997). Manifestations of chronic and acute stress in dogs. *Appl Anim Behav Sci 52*, 307-319.

Beerda, B., Schilder, M. B. H., Van Hooff, J. et al. (1999). Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. *Physiol Behav 66*, 233-242. doi:10.1016/S0031-9384(98)00289-3

Beerda, B., Schilder, M. B. H., Van Hooff, J. et al. (2000). Behavioral and hormonal indicators of enduring environment stress in dogs. *Animal Welfare 9*, 49-62.

Hetts, S. J., Clark, D., Calpin, J. P. et al. (1992). Influence of housing conditions on beagle behaviour. *Appl Anim Behav Sci 34*, 137-155.

Hsu, Y. and Serpell, J. A. (2003). Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *J Am Vet Med Assoc 223*, 1293-1300. doi:10.2460/javma.2003.223.1293

U.C. Davis. (2017, March 22). Animal adoptions. U.C. Davis Safety Services Procedure: *IACUC-36*. http://safetyservices.ucdavis.edu/article/animal-adoptions

VIII-428

Alternative methods for killing laboratory animals – For careful consideration in structurally departing from the prescribed methods

Marjolein Schilders-van Boxel, Frank Dales, Pieter Roelfsema, Herman Koëter, Henriëtte Bout, Jan-Bas Prins, Coenraad Hendriksen and Wim de Leeuw

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands

Marjolein.vanboxel@rvo.nl

In July 2016, the NCad (Netherlands National Committee for the protection of animals used for scientific purposes) presented its advisory report on "Alternative methods for killing laboratory animals" to the Dutch Minister of Agriculture, Martijn van Dam. The NCad was requested to advise on methods for killing laboratory animals that are considered to be at least as humane as the methods set out in European Directive 2010/63/EU. And to offer guidance to the Netherlands Food and Consumer Product Safety Authority (NVWA) in assessing such alternative methods of killing by providing elements that must comprise a scientific justification.

The Directive provides two possibilities for deviating from the prescribed methods of killing: 1) The purpose of the procedure cannot be achieved by the use of a method of killing set out in the Directive. The Central Authority for Scientific Procedures on Animals (CCD) can, on the basis of a scientific justification submitted by the applicant, decide to grant a project licence for a project in which a different method of killing is proposed than those set out in the Directive. The acceptance of such "divergent" methods of killing is limited to the specific research project for which the licence is granted. 2) The other method of killing is considered to be at least as humane as the appropriate methods set out in the Directive. On behalf of the Minister, the NVWA can, on the basis of a scientific justification submitted by the applicant, grant the establishment licensee an exemption or dispensation for a structural (i.e. outside-the-project) use of the alternative method of killing.

While the advisory report by the NCad focuses on the second option, it may also offer guidance for the CCD, as, if a researcher opts for a divergent method of killing for scientific reasons, the CCD will review whether that method is also acceptable from an animal welfare perspective.

For the purpose of assessing whether an alternative method of killing is at least as humane with regard to the individual animal as the current legally permitted methods, the NCad advises using the following elements: 1) speed of loss of consciousness; 2) degree of pain, suffering and distress associated with (the entire experience relating to) the killing. If it is intended to be used for groups of animals, the method of killing should be assessed on the basis of the individual animal within that group with the highest expected degree of pain, suffering and distress.

The NCad recommends performing the assessment of the alternative method of killing in the following way. The applicant for an exemption or dispensation submits to the NVWA, on the basis of a Synthesis of Evidence evaluation, data (also from the literature) demonstrating that with regard to the two elements stated above, the method is at least as humane as the current prescribed methods. This analysis should be based on relevant (or as relevant as possible) measurable parameters for and clinical observations (such as regarding behaviour) of the animals to which the application relates. Experts can compare those data with the available data for the prescribed methods of killing. If there are no data in the literature or a Synthesis of Evidence evaluation provides insufficient clarification for an assessment of the request for an exemption or dispensation, exploratory animal studies should be carried out in consultation with the NVWA (and after a project licence has been granted by the CCD), to add the missing data on the parameters relevant to welfare. The study (including "negative" results) is required to be published in an open access, peer-reviewed scientific journal, in accordance with the ARRIVE Guidelines. If the NVWA assesses favourably the data in the literature and a possible exploratory study, the NVWA can grant a dispensation for a defined period. The dispensation is granted subject to the condition that the applicant must first arrange for a scaled-up field trial to be conducted to ascertain the functionality of the alternative method of killing under the conditions that apply in practice (validate). As soon as the alternative method has been demonstrated to be at least as humane as the appropriate methods set out in the Directive, the NVWA should issue a generally applicable exemption for it.

The NCad recommends making centrally available the conditions for dispensation applied by the NVWA and data on the exemptions granted for alternative methods of killing. Knowledge sharing between the NVWA and CCD must be promoted, as well as between Animal Welfare Bodies (IvDs). And licensees should be aware of their obligation to have professionally competent employees.

Reference

https://english.ncadierproevenbeleid.nl/advice/documents/reports/16/9/15/ncad-opinion-on-alternative-killing-methods-for-laboratory-animals

VIII-429

Code of Practice on water and food deprivation in laboratory animals

Marjolein Schilders-van Boxel, Wim de Leeuw, Coenraad Hendriksen, Herman Koëter, Jan-Bas Prins, Henriëtte Bout, Reineke Hameleers, Pieter Roelfsema, Malou Wirken and Leane van Weereld

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands
Marjolein.vanboxel@rvo.nl

Although the method of restricting food and water for laboratory animals in neurocognitive research in the Netherlands is consistent with international practice, the Dutch Minister for Agriculture Martijn van Dam wants to look into possibilities to improve animal welfare within this method and/or exploring alternative methods. He therefore requested that the National Committee for the protection of animals used for scientific purposes (NCad) look into possible improvements to research methods with the aim of increasing laboratory animals' motivation to perform behavioural tasks. He asked the NCad to include in its research all species for which reduced food and water intake is used as a way of inducing them to perform tasks. The opinion includes best practices on rats and mice, and non-human primates (NHP), which are also disseminated in a European and international context.

This poster will give an overview of the advisory report by the NCad and the CoP.

Reference

https://english.ncadierproevenbeleid.nl/advice/contents/published-advisory-reports

VIII-458

Variability in rat behavior during exposure to CO_2

Lucia Amendola and Daniel M. Weary

Animal Welfare, University of British Columbia, Vancouver, BC, Canada
luciaame@mail.ubc.ca

Gradual-fill CO_2 is commonly used to kill laboratory rats but may cause fear, anxiety, and dyspnea in the time it takes for loss of consciousness to occur. Rats vary in their behavioral response to this agent, suggesting that some animals may find the procedure less aversive. The aim of the study was to assess individual variation in behavioral signs of distress during CO_2 exposure and to determine how this relates to individual differences in an aversion test. Eleven SD rats were exposed to CO_2 to assess their distress response during acute exposure, and exposed again in an aversion-avoidance test. Responses during forced exposure to CO_2 were negatively correlated with the degree of aversion to CO_2 (Pearson correlation test: $r = -0.55$, $p = 0.08$). These results indicate that rats vary in the degree to which rats find CO_2 aversive, suggesting that the welfare effects of this procedure vary depending upon individual characteristics.

VIII-614

Evaluation of X-ray properties of 3D printing materials for preclinical Positron Emission Tomography/ Computed Tomography (PET/CT) phantom development

Wendy McDougald[1], Nick Weir[2], Scott Inglis[3] and Adriana Tavares[1]

[1]Centre for Cardiovascular Sciences, University of Edinburgh, Edinburgh, United Kingdom; [2]Department of Medical Physics, NHS Lothian, University of Edinburgh, Edinburgh, United Kingdom; [3]Department of Medical Physics, NHS Lothian, Edinburgh, United Kingdom
s1575538@sms.ed.ac.uk

Traditionally, PET/CT phantoms reduced the number of animals used for imaging protocol optimization and contributed towards refinement of small animal scanning techniques. The development of novel anthropomorphic phantoms via 3D-printing technology can potentially replace animals all together when optimizing scanning protocols. Here we evaluate X-ray properties of 20 different 3D-printing materials. CT scans (low/high doses and default protocols) were acquired on a Mediso's PET/CT preclinical scanner. Measured Hounsfield Unit (HU) ranged from -60 to 230HU, -53 to 87HU and -158 to 62HU, low/high doses and default, respectively. Materials Endur and Tango Black Plus(T) yielded highest (62-230HU) and lowest (-53 to -60HU) HU, respectively. About 8% HU values were out of soft tissue acceptable range (e.g. muscle/adipose; -98 to +150HU) providing a basis for investigating materials aiming to mimic dense tissue (e.g. bone). Data supports the use of 3D materials for TEM phantom development.

VIII-616

Towards the replacement of fetal bovine serum in cell culture applications

Gilly Stoddart and Christopher Faßbender

PETA International Science Consortium Ltd., London, United Kingdom
gillys@piscltd.org.uk

Foetal bovine serum (FBS) used as a supplement for cell culture media presents significant scientific and animal welfare concerns. For example, considerable batch-to-batch variation exists and there is the risk of contaminating cells with problematic animal proteins and pathogens which can lead to unexpected outcomes. There are alternatives to the use of FBS that overcome these concerns.

Advances in biotechnological protein production allow for the production of recombinant proteins, and use of application-specific cell culture media supplements eliminates variability and biosafety issues, and eases product purification. In basic research and R&D testing, FBS can be replaced relatively easily; media recipes have been optimised for many cell types, although the concentration of supplements for some cell types will need further optimisation. There is also support for the use of serum-free media in regulatory testing.

This poster includes recommendations for the use of FBS alternatives in both regulatory and non-regulatory testing and information about companies that sell serum-free medium or cell culture supplements.

VIII-630

The long hard road to globally eliminating the chronic toxicity test in dogs

Patricia Bishop and Amy Clippinger

Regulatory Testing Department, People for the Ethical Treatment of Animals, Norfolk, VA, United States

PatriciaB@peta.org

Historically, most countries have required both a 90-day and a one-year chronic toxicity study be conducted on dogs for pesticide registration. Comparative analyses of data from the two tests, beginning in the late 1990s and continuing today, have repeatedly shown that the chronic study provides little additional relevant information. Accordingly, the United States removed the study from its pesticide data requirements in 2007, followed by the European Union in 2013, but years later, other countries still required the test. NGOs and industry have advocated for elimination of the chronic study requirement by providing regulatory agencies in these countries with supporting scientific evidence and urging them to harmonize with the US and EU. While most have since removed the test or are in the process of considering it, the length of time it has taken, in addition to each country's having to conduct its own analysis when abundant evidence was already available, is troubling and underscores the need to improve communication among countries and strengthen harmonization efforts to reduce animal use.

References

Dellarco, V. L., Rowland, J. and May, B. (2010). A retrospective analysis of toxicity studies in dogs and impact on the chronic reference dose for conventional pesticide chemicals. *Crit Rev Toxicol 40*, 16-23.

Kobel, W., Fegert, I., Billington, R. et al. (2010). A 1-year toxicity study in dogs is no longer a scientifically justifiable core data requirement for the safety assessment of pesticides. *Crit Rev Toxicol 40*, 1-15.

VIII-693

A systematic review of the welfare impact of carbon dioxide for euthanasia of laboratory mice and rats

Patricia Turner[1], Deborah Hickman[2], Tsutomu Kurosawa[3], Takashi Agui[3], Vera Baumans[4], Woo-Sung Choi[5], Yangkyu Choi[5], Paul Flecknell[6], Pedro Goya[7], Byeong Han Lee[5], Kathleen Pritchett-Corning[8] and Merel Ritskes-Hoitinga[4]

[1]University of Guelph, Guelph, ON, Canada; [2]Stark Neurosciences Research Institute, Indianapolis, IN, United States; [3]Osaka University, Osaka, Japan; [4]Division of Laboratory Animal Science, University of Utrecht, Utrecht, The Netherlands; [5]Korean College of Laboratory Animal Medicine, South Korea; [6]Newcastle University, Newcastle, United Kingdom; [7]Universitat Autonoma de Barcelona, Barcelona, Spain; [8]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States

pvturner@uoguelph.ca

There has been increased discussion about the use of CO_2 as a method for euthanizing laboratory rodents, including questions related to potential discomfort, pain or distress that animals may experience prior to loss of consciousness; time to loss of consciousness; best methods for use of this agent, as well as the suitability of this agent for rodent euthanasia and the availability of better alternatives. While these discussions have been useful in providing new information, they have resulted in significant confusion regarding the acceptability of CO_2 for rodent euthanasia, and in some cases, researchers and veterinarians have lost confidence in knowing which techniques to recommend or use for euthanasia of laboratory rodents in research settings. The International Association of Colleges of Laboratory Animal Medicine (IACLAM) conducted a systematic review of over 80 publications related to this subject using a SYRCLE registered systematic review protocol. The results of this work provide guidance on how to evaluate inhaled euthanasia options for rodents as well as the range acceptability of various procedures.

Theme IX – Global Cooperation

Coordinators
Carl Westmoreland, SEAC, Unilever, Bedford, United Kingdom
Hajime Kojima, Japanese Center for the Validation of Alternative Methods National Center for Biological Safety and Research, Setagaya, Tokyo, Japan

Oral Presentations

Session IX-1: Animal Welfare: A Global Perspective

Co-Chairs
Ekaterina Rivera, Universidade Federal de Goiás, Goiânia, Goiás, Brazil
Kathryn Bayne, AAALAC International, Frederick, MD, United States

IX-1-832

Animal welfare – A global perspective

Ekaterina Rivera
Universidade Federal de Goiás, Goiânia, Goiás, Brazil
e.rivera@uol.com.br

A review will be presented on how the Brazilian Law on Animal Experiments led to a better animal welfare in this country, demonstrating that the 3Rs were the basis of the Law. And how we dealt with Laboratory Animal Welfare before the Law was issued.

An overview of other Latin American countries will be presented, the ones which have laws related to laboratory animal welfare regulations also the ones that do not have, but are working for it. And some of them have no special laws, but their environmental laws cover laboratory animals.

IX-1-195

Global harmonization of research animal welfare through accreditation

Kathryn Bayne
AAALAC International, Frederick, MD, United States
kbayne@aaalac.org

Increasingly, scientific collaborations and contracts cross national borders. Bridging these international interactions through the assurance that animal research is conducted in a humane and conscientious manner is a clear scientific imperative for the reproducibility of research data and critical to ensure high quality research animal welfare. One way to mitigate the potential confounding effects the quality of the animals may have on the research data is to harmonize animal care practices and procedures worldwide to promote an environment of workplace integrity, ethics-based decision making, good communication of institutional expectations, clear lines of authority, and a system for continuous development and improvement of the animal care and use program. AAALAC International is in a unique position to harmonize animal care and use programs as, collectively, the expert teams that conduct the on-site evaluations visit more than 300 institutions each year across 42 countries.

IX-1-509

Achieving consistency in global standards for ethical review

Judy MacArthur Clark[1], Chang-Qing Gao[2] and Malcolm Macleod[3]
[1]JMC Consultancy, Sandwich, United Kingdom; [2]Laboratory Animals, Central South University, Changsha, People's Republic of China; [3]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom
judymacarthurclark@gmail.com

It is expected, internationally, that a statement is provided that published research involving animal studies has undergone ethical review. This may be used by journal editors and peer-reviewers, by co-authors, and by national and international bodies in judging the ethical status of the work. A number of transnational bodies (e.g. OIE, 2011) strongly recommend ethical review in their published guidance yet there is no agreed quality standard to which such review should adhere. As a result, the statements and guidance are of limited value.

Further, achieving global consistency of ethical review is hampered since no repository exists to enable the sharing of difficult or contentious decisions to help others faced with similar questions.

To achieve the necessary impact, any global standard for ethical review must be recognized by a range of internationally respected bodies. Further, an anonymized repository should be considered to enable bench-marking and consistency-checking.

In developing solutions, we can draw on experience of publishing human research as well as of delivering training in performing ethical review of animal research in diverse cultures.

Reference

OIE – World Organisation for Animal Health (2011). Bayne, K., Bayvel, D., MacArthur Clark, J. et al. The Use of Animals in Research and Education. In *OIE Terrestrial Animal Health Code* (Chapter 7.8). http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_aw_research_education.htm (accessed 21 April 2017)

IX-1-695

The changing role of the laboratory animal veterinarian – Results of an IACLAM survey

Patricia Turner[1], William White[2], Judy MacArthur Clark[3], Takashi Agui[4], Michele Bailey[5], Philippe Baneux[6], Yangkyu Choi[7], Rony Kalman[8], Seong Hyeok Seok[7], Je Kyung Seong[7] and Kazuo Yano[4]

[1]University of Guelph, Guelph, ON, Canada; [2]Veterinary and Professional Services, Charles River, Wilmington, MA, United States; [3]Animals in Science Regulation Unit, Home Office, London, United Kingdom; [4]Laboratory of Laboratory Animal Science and Medicine, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan; [5]Comparative Medicine, National University of Singapore, Singapore; [6]Cornell Center for Animal Resources and Education, Cornell University, Ithaca, NY, United States; [7]Department of Microbiology and Immunology, Seoul National University, Seoul, South Korea; [8]Authority for Biological and Biomedical Models, Hebrew University, Jerusalem, Israel

pvturner@uoguelph.ca

The International Association of Colleges of Laboratory Animal Medicine (IACLAM) conducted a 30-question survey of college members (ACLAM, ECLAM, JCLAM, and KCLAM) to evaluate the role of current board-certified laboratory animal veterinarians in research facilities around the world as well as areas where gaps existed in knowledge, which might impact research animal welfare. A total of 405 responses were received resulting in a response rate of at least 20% per college. Approximately 41% of respondents worked in academia and 21% in industry, and 82% of respondents worked full-time. A substantial amount of Diplomates' time (up to 50%) was devoted to administrative duties and animal ethics committee-related work with significantly less time devoted to hands-on animal-related activities, clinical work or research. Mice and rats were listed as the species consuming the most institutional resources, and in particular, genetically altered rodents. Diplomates provided further responses to questions related to rodent cage size, pain detection in rodents, euthanasia methods for rodents, and endpoint decision-making. These welfare results will be summarized.

IX-1-580

Global initiatives for replacement, refinement and reduction in animal use within pharmaceutical toxicology programmes

Helen Prior, Nikki Gellatly, Joanna Edwards and Fiona Sewell

NC3Rs, London, United Kingdom

helen.prior@nc3rs.org.uk

The UK's National Centre for the 3Rs (NC3Rs) convenes a wide range of international consortia to discuss opportunities to apply the 3Rs within (bio)pharmaceutical toxicology programmes. Expert working groups of industry scientists and regulators take an evidence-based approach to explore and validate new opportunities to minimise and refine the use of species including non-human primates, dogs and rodents. The NC3Rs acts as an honest broker to share pre-competitive clinical and non-clinical data to provide the basis for industry consensus opinions, recommending efficiencies in study designs and promotion of best practice.

Information on the following projects will highlight opportunities for reduction and refinements within studies: reducing the use of recovery animal groups to support first-in-human clinical trials, blood microsampling for toxicokinetics, social housing of non-rodents during telemetry recordings and reviewing the use of a second species within regulatory studies.

IX-1-435

The ethical use of animals in Brazil: Role of national legislation by CONCEA

Monica Levy Andersen

National Council for the Control of Animal Experimentation (CONCEA), Brazil

ml.andersen12@gmail.com

Animal experimentation has incited a great deal of debate, with a lot of the discussion focusing on ethical considerations. In 2008, Brazil emerged into a new era of animal research regulation, resulting in an increased focus, and rapid learning experience, on questions related to all aspects of animal experimentation. Law reinforces the idea that animal experiments must be based on ethical considerations and integrity-based assumptions. We aimed to provide an overview on the application of ethics in the use of animals in research and education in Brazil highlighting the role of National Council for the Control of Animal Experimentation (CONCEA) that published several guidelines. Based on validated protocols, 24 methods have already been recognized and after a period of 5 years of this recognition, the use of animals will be not allowed for those propositions. In the current Brazilian framework for regulating laboratory animal science, CONCEA has provided marked advance in improving the ethical and legal rules for the welfare of the animals that resulted in the awareness of the scientific community for their use in research or education purposes.

Session IX-2: International Approaches to Validation

Co-Chairs
Valérie Zuang, European Commission, Joint Research Centre, Italy
Hajime Kojima, National Institute of Health Sciences, Tokyo, Japan
Warren Casey, NTP, Research Triangle Park, NC, United States

IX-2-368

JaCVAM update

Hajime Kojima and Akiyoshi Nishikawa

Japanese Center for the Validation of Alternative Methods (JaCVAM), Biological Safety Research Center (BSRC), National Institute of Health Sciences (NIHS), Tokyo, Japan
bibi200409@yahoo.co.jp

Since 2005, JaCVAM has contributed to establish several OECD Test Guidelines (TGs) and Guidance documents. In 2016, they are OECD Test No. 442E: h-CLAT assay for skin sensitization testing, OECD Test No. 458: Stable transfected transcriptional activation (STTA) assay for the detection of androgen agonist & antagonist (AR-Ecoscreen) and OECD Series on Testing & Assessment No. 231: Guidance Document on the *In Vitro* Bhas 42 Cell Transformation Assay. In the OECD Work plan, Japan has proposed five test methods: 1) the IL-8 Luc assay for skin sensitization testing, 2) Hand1-Luc EST (Embryo Stem cell Test) for the developmental screening and 3) ROS (Reactive Oxygen Species) assay for photosafety, 4) Vitrigel-EIT (Eye Irritation Test) for the eye irritation testing and 5) LabCyte Cornea model-EIT for the eye irritation testing. Through these activities, Japan has played a role to develop the alternative to animal testing for safety evaluation of chemicals.

IX-2-738

Update from the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Warren Casey

NTP, Research Triangle Park, NC, United States
warren.casey@nih.gov

The mission of ICCVAM, which is composed of representatives from 16 U.S. federal regulatory and research agencies, is to promote the regulatory acceptance of test methods that protect human and animal health and the environment while reducing, refining, or replacing the use of animal tests. This presentation will provide an update on ICCVAM's (1) approach to exploring new paradigms for the validation of alternative toxicological methods, (2) areas of priority and scientific focus for immediate resource investment, (3) new initiative to provide alternatives (replacement) for the six most commonly used acute toxicity tests ("EPA 6-Pack") and (4) efforts in coordinating a new Strategic Roadmap to establish new approaches for evaluating the safety of chemicals and medical products in the United States

IX-2-513

Brazilian experience on innovation, validation and regulation of alternative methods to animal use

Isabella Delgado[1,2]

[1]INCQS, Fiocruz, Rio de Janeiro, RJ, Brazil; [2]RENAMA, Brasília, DF, Brazil
delgadoisabellaf@gmail.com

Since 2012, Brazil counts on the National Network of Alternative Methods (RENAMA), which is set up under the Ministry of Science, Technology, Innovation and Communication (MCTIC) and supervised by a steering committee. RENAMA is composed by 3 central laboratories, the Brazilian Center for Validation of Alternative Methods (BraCVAM), 33 associated laboratories, and interacts with regulatory agencies and industry. It is mainly involved in the validation study process, as well as demonstrating the applicability of already validated methods. The main actions of RENAMA are (i) Identify technical competence and promote the quality of the biological inputs used by the laboratory network; (ii) Disseminate alternative methods in Brazil and Mercosur via support, training and distribution of technical protocols (translated by RENAMA's technical-scientific coordination); (iii) Provide reference material (certified or not) for the establishment of interlaboratory tests; and (iv) Stimulate and promote the development and validation of new alternative methods.

References

ANVISA (2015). Resolution RDC No 35/2015 on acceptance of alternative methods of 7 August 2015. Diário Oficial da União. N° 151, Seção 1. p.44. http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=44&data=10/08/2015 (accessed 7/04/17)

CONCEA (2014). Normative Resolution No 18/2014 on alternative methods of 24 September 2014. Diário Oficial da União. N° 185, Seção 1. p.9. http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=25/09/2014&jornal=1&pagina=9&totalArquivos=120 (accessed 5/04/17)

CONCEA (2016). Normative Resolution No 31/2016 on alternative methods of 19 August 2016. Diário Oficial da União. N° 160, Seção 1. p.4. http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=4&data=19/08/2016 (accessed 6/04/17)

MCTIC (2012). Ordinance N°491/2012 of 03 February 2012. http://renama.org.br/ (accessed 21/04/17)

IX-2-297

KoCVAM activities for the development and dissemination of alternative test methods

Jong Kwon Lee[1], Tae Sung Kim[1], Il Young Ahn[1], Eun-kyung Ku[1], Ji-Sun Lee[1], Kyungyuk Ko[1], Jung-Sun Yi[1], Ji Young Kim[1], Joohwan Kim[1] and Hye-Kyung Park[2]

[1]Toxicological Screening & Testing Division, National Institute of Food and Drug Safety Evaluation, South Korea; [2]Toxicological Evaluation and Research Department, National Institute of Food and Drug Safety Evaluation, South Korea

jkleest@korea.kr

The Korean Center for the Validation of Alternative Methods (KoCVAM) was founded by the Ministry of Food and Drug Safety (MFDS) in 2009 with the aim of developing and promoting alternative test methods and ultimately enhancing public health and animal welfare. In 2011, KoCVAM joined the International Cooperation on Alternative Test Methods (ICATM) by signing a Memorandum of Cooperation with four international agencies, ICCVAM, EURL ECVAM, JaCVAM and Health Canada. KoCVAM has been committed to developing and disseminating alternative test methods in Korea. KoCVAM developed an alternative test method named LLNA: BrdU-FCM, which evaluates skin sensitization potential of chemicals, and proposed the assay to the OECD. KoCVAM has also adopted 13 OECD Test Guidelines and provided industry, academia and other agencies with educational programs and technical transfer activities through workshops in Korea. KoCVAM will continuously play a pivotal role in further promoting the use of alternative test methods and contribute to developing internationally harmonized alternatives to animal testing in collaboration with ICATM partners.

IX-2-269

EURL ECVAM's activities to reach global acceptance and use of alternative methods and approaches

Valérie Zuang

European Commission, Joint Research Centre, Ispra (VA), Italy

valerie.zuang@ec.europa.eu

The mandate of EURL ECVAM is defined in EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Its key responsibilities are to coordinate and promote the development and use of alternatives; coordinate the validation of alternative approaches at EU level; act as a focal point for the exchange of information on the development of alternative approaches; set up, maintain and manage public databases and information systems on alternative approaches and; promote dialogue between legislators, regulators, and all relevant stakeholders in view of the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.

This presentation will provide an update on EURL ECVAM's activities addressing these key provisions with particular emphasis on international cooperation with a view to accelerate world-wide adoption of alternative methods.

References

EU (2010). Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J Eur Union L 276*, 33-79.

Zuang, V. et al. (2016). EURL ECVAM Status Report on the development, validation and regulatory acceptance of alternative methods and approaches (2016). *JRC Report EUR 28156 EN*. http://publications.jrc.ec.europa.eu/repository/

IX-2-28

Inter-laboratory study validation of *in vitro* toxicity and antigenicity assays for *Clostridium septicum* vaccine antigens

Botond Siklodi[1], Keith Redhead[2*], Arnold Daas[3], David Le Tallec[3], Lukas Bruckner[4] and Marie-Emmanuelle Behr-Gross[3]*

[1]Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Hungary; [2]Vaccine & Assay Consultancy Ltd., United Kingdom; [3]Council of Europe EDQM, France; [4]Switzerland

botond.siklodi@ceva.com

In 2016, 14 manufacturers and public-sector control laboratories enrolled into an international study run under the common aegis of the European Partnership for Alternative Approaches to Animal Testing (EPAA) and the European Directorate for the Quality of Medicines and HealthCare (EDQM).

The study aims at validating Vero cell-based alternatives to the mouse tests currently in use for in-process quality control of *Clostridium septicum* vaccines (toxicity: Minimum Lethal Dose, antigenicity: Total Combining Power). Previous studies (Redhead et al., 2011; Daas et al., 2017) demonstrated good repeatability and reproducibility, increased sensitivity and excellent concordance with the corresponding *in vivo* tests for the Vero cell-based toxicity and antigenicity assays.

The preliminary results of the present study on optimised Vero cell-based assays confirm that the latter are credible alternatives to the *in vivo* methods and suggest that implementation of cell-based testing for other cytotoxic antigens, using this study as a model, could ultimately result in large reduction of animal usage in the quality control of veterinary vaccines.

[*]*contributed equally*

Reference

Seventh Report from the Commission to the Council and the European Parliament on the Statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union COM(2013)859/final (Consulted on 20.05.2015)

Session IX-3: International 3Rs Cooperation: The Role of International Science

Co-Chairs
Tsutomu Kurosawa, Kagoshima University, Sapporo, Japan
Carl Westmoreland, SEAC, Unilever, Bedford, United Kingdom

IX-3-330

A global campaign to end animal testing of cosmetics via the United Nations

Katy Taylor and Michelle Thew
Cruelty Free International, London, United Kingdom
katy.taylor@crueltyfreeinternational.org

Since the EU 2013 testing ban, ten other countries have enacted some form of restrictive legislation on testing cosmetics on animals – some more comprehensively than others. There is a promised ban in Australia and one under consideration in Canada. But waiting for country after country to end unethical, outdated and unnecessary cosmetics animal testing is a slow process. Eighty per cent of countries would still allow this testing, yet there is limited scientific justification for it and strong public opinion against it. Cruelty Free International has launched a campaign for a global end to animal testing of cosmetics via the UN. A UN resolution starts with one UN member state tabling a resolution to the General Assembly and championing it so that it remains on the agenda, gets debated and wins majority support. For that to happen, the public, campaigners, retailers, industry, regulators and scientists must work together to bring pressure to bear on decision-makers the world over.

IX-3-521

3Rs for the development of medical devices and cell therapies

Tsutomu Kurosawa
Veterinary School, Kagoshima University, Chuo-ku, Sapporo, Japan
japanawr@gmail.com

Medical device industries have used laboratory animals in the early stage of the development for handicapped and ill human patients. Alternative science for the medical device development has been progressed using idea raised by pharmaceutical and cosmetic industries. ISO has set various standard documents for biological and clinical evaluation of medical devices at TC194. There are many testing methods and the most of these require laboratory animal testing. However, ISO/TC194 WG3 for animal protection aspect has published "Animal Welfare Requirement" as ISO10993-2. This IS well states the importance of 3Rs. For instance, an international round robin test with Episkin and Epiderm for skin irritation and sensitization test has been conducted by ISO/TC194 WG8 alternative to Guinea Pig skin test accord with ISO10993-2. The international standard for animal welfare requirement can be applied to the biological evaluation of stem cell therapies which is developed rapidly.

IX-3-204

Cellular stress, tipping points and application in risk assessment: An international research programme

Carl Westmoreland
SEAC, Unilever, Bedford, United Kingdom
carl.westmoreland@unilever.com

An important aspect to the use of Adverse Outcome Pathways for making decisions about human safety is that they must be quantitative and any predictions relating to dose must be interpretable in the context of actual levels of human exposure. Understanding both dosimetry and PK modelling have been identified as key components relating to the use of *in vitro* data in "Next Generation" risk assessments.

We are working with scientific partners from around the globe (US, China, UK, India, Netherlands and Ireland) to investigate the concentration-dependent transition from adaptation to injury for several different stress responses (oxidative stress, mitochondrial toxicity, DNA damage). Understanding *in vitro* concentrations that relate to this "tipping point" between adaptation and adversity and how ultimately this information can be used in consumer safety risk assessment is a scientific challenge that can only be addressed with truly collaborative, multi-disciplinary research.

IX-3-133

International 3Rs effort for pharmaceuticals and other products

Abigail Jacobs
CDER, US FDA, Silver Spring, MD, United States
abigail.jacobs@fda.hhs.gov

International cooperation, alone, through the International Council for Harmonization (ICH) guidelines for development of pharmaceuticals, such as ICHM3R2-product development and timing, ICHS6-biologics development, ICHS3 Q and A-microsampling reduces animal use by elimination of worldwide repetition of studies, in addition to providing specific alternative approaches to addressing various assessments. Many OECD stand-alone nonanimal alternatives have been developed for assessment of local toxicity (skin, eye, and photo). However, assessments of systemic effects currently being worked on (e.g., embryo-fetal malformation, and carcinogenicity) are more challenging, and a suite of assessments will probably be needed to address those effects. OECD documents provide guidance on the Use of adverse outcome pathways in developing integrated approaches to testing and assessment.

IX-3-650

Trans-Atlantic partnerships are key to advancing TT21C: Example case studies on defining adversity *in vitro*

Rebecca Clewell[1], Yeyejide Adeleye[2], Andrew White[2], Paul Carmichael[2], Salil Pendse[1], Patrick McMullen[1] and Melvin Andersen[1]

[1]ScitoVation, Durham, NC, United States; [2]Safety and Environmental Assurance Centre, Unilever, United Kingdom
rclewell@scitovation.com

International research collaborations ensure *in vitro* tools and strategies are applicable to regulatory agencies world-wide. To this end, ScitoVation and Unilever have been working together to identify the cellular processes that drive chemical dose-response and define the tipping point for adversity *in vitro*. Two case studies will be presented: the DNA damage and oxidative stress response pathways. An AOP approach was used to develop *in vitro* assays, and in-depth dose-response data were collected for each key event. At low concentrations, post-translational processes (formation of DNA repair centers, induction of antioxidant activity) efficiently mitigate cellular stressors. Transcriptional responses occurred at higher concentrations than those causing damage and post-translational responses, providing mechanistic support for threshold-shaped dose-response curves. Together, this work supports the transition to quantitative *in vitro* based safety assessments by defining regions of safety for chemical exposures.

IX-3-684

Enhancing the application of alternative methods through global cooperation

Russell Thomas

National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC, United States
thomas.russell@epa.gov

Progress towards the development and translation of alternative testing methods to safety-related decision making is a common goal that crosses organizational, stakeholder, and international boundaries. The challenge is that different organizations have different missions, different regulatory frameworks, and need to apply alternative methods to different decision contexts. Advancing the development and application of alternative methods will require focusing on common goals that address key challenges in advancing toxicology testing in the 21st century and provide common benefit across organizations and international boundaries. The talk will describe the global cooperation activities by the EPA for development and translation of alternative testing methods and the lessons learned.

This abstract does not necessarily reflect U.S. EPA policy.

IX-3-491

Exploring the value of new approach methodologies in read-across: The parabens as a collaborative EU-ToxRisk – Cosmetics Europe case study

Gladys Ouedraogo[1], Bart Van der Burg[2], Catherine Mahony[3], Jorge Naciff[4], Corie Ellison[4], Ann Detroyer[1], Dagmar Bury[1], William Drewe[5], Tony Long[5], Hennicke Kamp[6], Dinant Kroese[7], Sylvia Escher[8], Tom Cull[9], Andrew White[9], Matthew Dent[9], Bas Blaauboer[10], Detlef Keller[11], Egon Willighagen[12], Mark Cronin[13], Richard Currie[14], Rabea Gräpel[15], Bob Van de Water[15] and Enrico Mombelli[16]

[1]L'Oréal R&I, Aulnay sous Bois, France; [2]Biodetection Systems, Amsterdam, The Netherlands; [3]P&G, Bagshot, United Kingdom; [4]P&G, Cincinnati, OH, United States; [5]Lhasa Limited, Leeds, United Kingdom; [6]BASF, Ludwigshafen, Germany; [7]TNO, Zeist, The Netherlands; [8]Fraunhofer Item, Hannover, Germany; [9]Unilever, Bedford, United Kingdom; [10]Institute For Risk Assessment Science, Utrecht, The Netherlands; [11]Henkel, Düsseldorf, Germany; [12]Maastricht University, Maastricht, The Netherlands; [13]University of Liverpool, Liverpool, United Kingdom; [14]Syngenta, Berkshire, United Kingdom; [15]University of Leiden, Leiden, The Netherlands; [16]Ineris, Verneuil-en-Halatte, France
gouedraogo@rd.loreal.com

Both EU-ToxRisk – a Horizon 2020 flagship project – and Cosmetics' Europe Long Range Science Strategy aim at developing pragmatic approaches for performing safety assessment and fostering their regulatory acceptance. Towards these goals, case studies are key in delivering new science by exploring new approach methodologies and challenging the decision-making frameworks used to run them. EU-ToxRisk and Cosmetics Europe agreed to work on the parabens to address these goals and optimize existing read-across templates.

This case study will allow us to establish a proof-of-concept for value added by new approach methodologies in read across: use of *in vitro* toxicodynamic and biokinetic data (like mechanistic assays, transcriptomics, protein binding, *in vitro* metabolism and liver clearance) for safety assessment, including consideration of aggregate exposure. Short chain parabens will be used to read-across longer chain ones and the exercise will then be extended to branched molecules.

IX-3-439

A*STAR Singapore: Innovating alongside regulators, 3Rs organisations, and industry for global impact

Kenneth Lee

Agency for Science, Technology and Research, Singapore
sherlyn_oh@bmsi.a-star.edu.sg

The Agency for Science, Technology and Research (A*STAR) is Singapore's lead government agency for innovation. Within a framework of responsible innovation, and drawing on our multi-disciplinary capabilities spanning life sciences, physical-chemical sciences, and engineering, we are building a programme to help advance the science of chemical safety assessment with fit-for-purpose, non-animal tools. We work closely with international regulators to validate and accelerate the adoption of novel approaches for safety assessment, and have on-going collaborations with the US EPA to address current gaps in toxicity testing. In an international case study with the US EPA, Health Canada, ECHA, and EFSA, we are examining the utility of our imaging-based machine learning technology for automated analysis and classification of cellular phenotypes in risk assessment. With the NC3Rs, the largest funder of 3Rs research in the UK, we are embarking on the first international CRACK IT Challenge to drive innovation and deliver 3Rs benefits through joint projects with industry and EU-based scientists. Examples of how we partner with food and consumer care multinational corporations to develop predictive tools for toxicity will also be illustrated.

Session IX-4: Global Regulatory Updates

Co-Chairs
Ellen Fritsche, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
Chantra Eskes, SeCAM, Magliaso, Switzerland

IX-4-744

Regulatory changes and impacts for 3Rs science: A case study for developmental neurotoxicity

Ellen Fritsche

IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
ellen.fritsche@uni-duesseldorf.de

The National Academy of Sciences Report from 2007 provided reasons and a roadmap for the paradigm shift in toxicology: moving from apical endpoint testing in animals to a pathway-based hazard and risk assessment by making use of computational as well as alternative *in vitro* and *in vivo* strategies. This paradigm shift has been reaching the academic, industrial and regulatory community. However, there is a strong need for international regulatory as well as academic harmonization when animal testing should be replaced by alternative testing strategies. Moreover, common testing standards are crucial across nations to limit resources needed for safety testing. The OECD together with other regulatory agencies play crucial roles in such harmonization processes. Here, a "case study" for such a development will be presented that describes the path that alternative testing for developmental neurotoxicity (DNT) has been taken over the last decade on the way to regulatory implication.

IX-4-262

Use of the Porcine Corneal Opacity and Reversibility Assay (PorCORA) for testing detergent and cleaning products identified as *in vivo* Category 1 (Cat. 1) due to persistence of ocular tissue damage according to the UN Globally Harmonized System (GHS) of classification

Chantra Eskes[1], Caroline Bertein[2], Micheal Carathers[3], George DeGeorge[3], Martina Hermann[4], Penny Jones[5], Pauline McNamee[6] and Bennett Varsho[3]

[1]SeCAM, Magliaso, Switzerland; [2]International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.), Brussels, Belgium; [3]MB Research Labs, Spinnerstown, PA, United States; [4]Henkel AG & Co. KGaA, Düsseldorf, Germany; [5]Unilever Safety and Environmental Assurance Centre, Bedford, United Kingdom; [6]The Procter & Gamble Company, Egham, United Kingdom
chantra.eskes@secam-ce.eu

Currently adopted OECD test methods for eye hazard do not allow identification of UN GHS Cat. 2 chemicals. A key reason is that these assays may not cover all relevant mechanisms of ocular damage. In particular, test methods adopted to identify UN GHS Cat. 1 were not designed to predict chemicals classified *in vivo* due to tissue effects

persisting 21 days after exposure. In contrast, PorCORA was designed to specifically address (ir)reversibility of corneal damage in *ex vivo* porcine corneas cultured for 21 days. Detergent and cleaning products having a balanced distribution of Cat. 1 / Cat. 2 / No Cat. based on existing *in vivo* data were tested using PorCORA with an extended washing procedure. All 5 *in vivo* UN GHS Cat.1 products based on persistence of effects, as well as 9 of 12 *in vivo* non-Cat. 1 products were correctly identified by the assay. PorCORA appears therefore as a promising assay for further investigation within e.g. Defined Approaches for eye hazard identification.

IX-4-605

Challenges in implementing the Frank R. Lautenberg Chemical Safety for the 21st Century Act: A perspective covering stakeholders, the U.S. Congress and the current administration

Sara Amundson

Humane Society Legislative Fund, Washington, DC, United States
samundson@hslf.org

After decades of discussions and truncated attempts to legislate, the 1976 U.S. Toxic Substances Control Act (TSCA) was reauthorized in the 114th Congress by overwhelming margins in the U.S. House and Senate. Bill language to require the use of existing alternatives to animal testing where available for both registrations and voluntary testing, ensuring that other strategies and methodologies are considered prior to animal testing and prioritizing research and development of new methods was negotiated as part of a final package passed by the U.S. House by a margin of 403-12. However, jockeying between stakeholders on implementation, a proposal by the Trump Administration to slash the Environmental Protection Agency's (EPA) budget by 31% for fiscal year 2018, and concerns over ensuring the tools and budget exist to meet the 21st century science paradigm to reduce, and ultimately eliminate, the use of animals for data generated for risk assessments have created significant dialogue. This dialogue must result in a concerted effort to protect the EPA's research and development budget, encourage implementation of existing strategies, and prioritize the nonanimal fundamentals in statutory language that facilitate assessing the risk of chemicals regulated under TSCA.

IX-4-131

Collaborative industry and regulatory efforts to implement alternative methods in China

Erin Hill, Quanshun Zhang and Rodger Curren

Institute for In Vitro Sciences, Gaithersburg, MD, United States
ehill@iivs.org

Although many companies have not used animals for safety assessment of cosmetics for decades, some international regulatory authorities still require animal testing for the registration and importation of cosmetics. While there are differing hurdles to acceptance of non-animal methods around the world, a common difficulty is lack of technical training and experience in interpreting *in vitro* data. IIVS has organized a group of companies to form the Industry Council for the Advancement of Regulatory Acceptance of Alternatives (ICARAA) which provides support of IIVS' mission to increase the use of *in vitro* methods internationally. Many of ICARAA's activities are currently in China where there is interest on the part of the regulatory authorities to understand and adopt non-animal approaches to substantiate safety of cosmetics. Specific examples of the programs developed for China will be presented and resulting successes and challenges will be explored.

IX-4-460

Implementation of guidelines to replace animal testing is not the end of the story

Barbara Birk, Bennard van Ravenzwaay and Robert Landsiedel

Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany
barbara.birk@basf.com

New methods replacing animal testing have considerably increased in the last years. Significant efforts were done in the development, establishment and validation of new alternatives leading to new OECD Guidelines. Nevertheless, the implementation of new guidelines is not the end of the story – harmonized assessment of the data, regulatory acceptance and the training of the different stakeholder (CROs, academia, regulatory) is mandatory to finally replace animal studies in a meaningful and efficient way. BASF is furthering the use of alternative methods by performing in-house development and -validation as well as gathering and assessing post-validation data to 1) define the potential and limitations of methods and testing strategies 2) discussing the results with regulatory agencies in the registration process and 3) offering trainings to interested stakeholder (IIVS-, EU-NETVAL-, CAAT-workshops). A sustainable success can only be achieved with intensive collaboration together.

IX-4-282

International regulatory test guidelines (TGs): Initiative for implementing serum free culture media

Horst Spielmann
FU Berlin, Berlin, Germany
horst.spielmann@fu-berlin.de

Due to significant progress in cell and tissue culture, animal test in pharmacology and toxicology have been replaced by *in vitro* culture methods based on human and animal cells, tissues and organs.

Fetal calf serum (FCS) or newborn calf serum (NCS) are essential ingredients of culture media, although for scientific and ethical reasons such media should be replaced by serum free media. Almost all international regulatory test guidelines (TGs), e.g. ICH, OECD and EU TGs, which are based on *in vitro* culture of human and animal cells and tissues, only recommend the use of FCS and NCS. Moreover, they recommend to avoid variability "a sufficient amount of NCS should be reserved" rather than switching to serum free media, e.g. the NRU *in vitro* phototoxicity test (ICH S10, OECD TG 432) and the validated embryonic stem cell test EST to predict embryotoxicity *in vitro* (Nature Protocols, 2011). Thus, in order to terminate the use of serum in culture media, international regulatory agencies and the scientific community should encourage research to replace the use of FCS and NCS by serum free media in their test guidelines.

IX-4-454

Issues associated with intellectual property in OECD Test Guidelines

Anne Gourmelon and Patience Browne
OECD, Paris, France
anne.gourmelon@oecd.org

In the last years, a number of OECD Test Guidelines based on *in vitro* techniques have been adopted while they contain protected elements. One important aspect of OECD Test Guidelines as regulatory standards in the context of chemical safety is their broad availability for use, their clarity and transparency, the consistent supply of all critical elements of the test method to ensure results from these alternative test methods can meet the goal of Mutual Acceptance of Data. There is also a monetary benefit for test methods that are included in a regulatory test guideline and OECD recognizes the need to encourage innovation and promote novel test method development. Recent experience with some protected elements reinforces the need to develop guiding principles around availability of test methods, the acceptable degree of transparency around IP coverage, the promotion of good licensing practices, and the supply of quality material intended to be used as regulatory standards.

IX-4-157

Latest activities and future directions of JSAAE for Asian cooperation toward 3Rs

Yasuyuki Sakai[1] and Hajime Kojima[2]
[1]Department of Chemical System Engineering, University of Tokyo, Tokyo, Japan; [2]Japanese Center for the Validation of Alternative Methods, National Institute of Health Sciences, Tokyo, Japan
sakaiyas@iis.u-tokyo.ac.jp

After the official establishment in 1990, the Japanese Society for Alternatives to Animal Experiments (JSAAE) has grown up to have about 450 members as of 2017. While promoting 3Rs research in Japan through a wide variety of activities, we have been making serious efforts to various aspects of international cooperation and contributions to 3Rs, such as hosting 6th World Congress on Alternatives and Animal Use in Life Science in Tokyo (WC6, 2007), or contributing setting up of various guideline/guidance with intimate cooperation with Japanese Center for the Validation of Alternative Methods (JaCVAM) etc. JSAAE now have cooperation agreements with counterpart societies in Korea, EU and USA and also is planning to set up with Chinese counterparts. We recently organized Asian Congress 2016 on Alternatives and Animal Use in the Life Sciences, November, 2016 in Japan under the sponsorship of the Alternatives Congress Trust (ACT). Total attendees of the congress were 400 and it was the first conference of its kind for researchers from Asia where the concept of the 3Rs is just now achieving penetration.

Session IX-5: Global Regulatory Harmonization

Co-Chairs
Cheng Jun, Guangdong Inspection and Quarantine Technology Center, Guandong, People's Republic of China
Anne Gourmelon, OECD, Paris, France

IX-5-182

Middle-out way to enlarge consensus of alternative to animal testing in China

Cheng Shu Jun[1], Qin Yao[2], Xu JiaTing[2] and Geng Meng Meng[2]

[1]Guangdong Inspection and Quarantine Technology Center, Guangdong, People's Republic of China; [2]In Vitro Science Lab, Guangzhou CHN-ALT Biotech Co. Ltd, Guangzhou, People's Republic of China

chengsj@126.com

The need of mutual acceptance of data and harmonization of global market have made alternative to animal testing an irresistible general trend for worldwide countries to made regulation and policy reform. Recent 10 years, we put forward a middle-out strategy to promote the development of cosmetics AAT in China. The middle-up way including standardization of *in vitro* methods, engaging in technology research and innovation in practice, improving the translation efficiency of toxicity test of AAT. The middle-out way is to outspread the AAT on multi-industries especially cosmetics and detergent by building up implementation capability, promote the forming of lab network, followed the accreditation criterion of CNAS, popularization *in vitro* bioscience, academic exchange and hand-on training. Chinese Conference of Alternative methods has held seven times. Most of the methods listed in the OECD test guidelines have been used by testing institutions and industries. More and more cosmetics companies want to set up *in vitro* lab by themselves and to accept *in vitro* data. In partnership with global collaborators across governments, academia, and industry, China is in critical crossroad to take the opportunity to develop modern alternative methods, shift to the toxicity paradigm and regulations fully accept AAT.

IX-5-448

Understanding the benefits of global regulatory harmonization: Experience at OECD

Anne Gourmelon

OECD, Paris, France

anne.gourmelon@oecd.org

With the blooming of validated alternatives to animal testing that are available to the regulators and the regulated community, harmonisation of their use becomes very important to avoid exponential growth of testing requirements across countries. A lot of unnecessary testing could be avoided if there was a better understanding of which alternative method or combination of, to select in defined situations. Dialogue, exchange of data and information, and agreements on common decision schemes across countries are essential steps towards global harmonization. Harmonisation brings economic benefits and saves time and resources, including animal lives. Indeed, not all countries are yet on the same page regarding the use of alternatives, and for manufacturers to place chemicals on the market, in some cases the whole range of possible assays (*in vivo* and *in vitro*) may still have to be performed to comply with countries' very different data requirements. Hence global harmonisation remains a relevant and necessary goal.

IX-5-309

Ensuring equal standards and adherence to the 3Rs in an increasing global environment

Kirstine Øvlisen

3R Strategy & Veterinary Services, Novo Nordis A/S, Måløv, Denmark

saq@novonordisk.com

At present, research using living animals is essential for all pharmaceutical companies in the discovery, development and production of new pharmaceutical and medical products.

We recognise our responsibility when working with animals in research and the welfare of the animals is given high priority. Global standards following the Directive 2010/63/EU have been set and the principles of the 3Rs are integrated into our processes and procedures. Working in an increasingly global environment requires a strong set-up and a supporting framework to ensure adherence and continuous improvements of equal high standards across sites.

In this presentation, an overview of the way animal welfare and the 3Rs are embedded in and governed by Novo Nordisk globally will be given.

IX-5-235

Towards global harmonisation of 3Rs in biologicals: Deletion of GST for vaccines

Katrin Schutte[1] and Anna Szczepanska[2]

[1]European Commission, Brussels, Belgium; [2]European Federation of Pharmaceutical Industries and Associations (EFPIA), Ixelles, Belgium
katrin.schutte@ec.europa.eu

The European Partnership for Alternative Approaches to Animal Testing (EPAA) project team on Global Harmonisation of 3Rs in Biologicals convened an international workshop in 2015 which achieved consensus to actively encourage the deletion of general safety tests (GST, e.g. abnormal toxicity tests in mice or guinea pigs, target animal batch safety tests) for vaccines from legal requirements and guidance documents, such as pharmacopoeia monographs, WHO recommendations, and OIE guidelines.

These tests have become obsolete through introduction of Good Manufacturing Practice and use of adequate and stringent quality control measures. Advanced process understanding, in-process controls, validation of the manufacturing process and release testing complying with international standards are also part of modern vaccine development and render the general safety test in animals obsolete.

The presentation will summarise the scientific background to the recommendations and key conclusions of the workshop and will report on the important steps achieved since towards deletion of the general safety tests at the level of the European Pharmacopeia, WHO and OIE.

Reference

https://circabc.europa.eu/sd/a/4a081e45-f19f-47f7-8d8d-65f4f10fccff/ihb%20sept%202015%20report.pdf

IX-5-110

Industry and regulatory collaboration to help modernize the US EPA 6 pack: Revised prediction model of the Skin Irritation Test (SIT, OECD TG 439) to predict EPA hazard categories

Rodger Curren, Erin Hill, Devin Sheehan and Gertrude-Emilia Costin

Institute for In Vitro Sciences, Inc., Gaithersburg, MD, United States
rcurren@iivs.org

We evaluated the validated *in vitro* SIT to determine if it can be used for EPA dermal hazard category assignment. Based on the Global Harmonized System (GHS), the SIT separates Category 2 skin irritants from non-irritants (No Category) using a single: exposure (60 min.) to the EpiDerm™ model (MatTek Corp.), post-exposure (42 h) and cut-off value of 50% tissue viability. Our retrospective analysis of paired *in vivo-in vitro* data (41 chemicals) showed that the SIT prediction model (PM) did not adequately predict EPA hazard categories. We revised the PM using two exposures (15 and 60 min.) and post-exposures (24 and 42 h) and a cut-off value of 20% viability. Preliminary testing of a subset of chemicals from the group of 41 showed that the proposed EPA PM could identify Categories II and IV accurately, and Category III with 46% sensitivity. We are currently investigating a larger and more diverse set of chemicals to test the validity of this EPA PM for dermal hazard labeling.

IX-5-450

Alternatives to animal testing in the OECD Test Guidelines Programme

Patience Browne and Anne Gourmelon

Test Guidelines Programme, OECD, Paris, France
patience.browne@oecd.org

The OECD Test Guidelines Programme provides tools for assessing chemical effects on human health and the environment. Test guidelines are internationally accepted, standardized methods and data generated from test guidelines following principles of Good Laboratory Practices are covered by Mutual Acceptance of Data, meaning results are accepted by OECD member and partner countries for evaluating chemical safety. In the last decade, new technology has been developed for chemical screening, yet international standards for safety screening continue to be largely a checklist of studies conducted in animals. OECD is taking efforts to promote increasingly sophisticated approaches for developing alternative test and non-test methods and evaluating performance of novel approaches. In 2012, OECD launched the Adverse Outcome Pathways Knowledge Base as a repository for information and research tool, and in 2016, published guidance on AOPs use for developing integrative testing strategies. Further, OECD is striving to develop alternatives to animal tests that are amenable to standardization and can be broadly used to enable mutual acceptance of data.

IX-5-503

Deletion of scientifically redundant animal test requirements in the agrochemical sector: The case of the 1-year dog study

Marco Corvaro[1], Troy Seidle[2], Thomas Hartung[3,5], Catherine Willett[4], Joanne Zurlo[5], Sean Gehen[6] and Jyotigna Mehta[1]

[1]Dow AgroSciences, Hitchin, United Kingdom; [2]Humane Society International, Montréal, Canada; [3]CAAT-Europe, University of Konstanz, Konstanz, Germany; [4]The Humane Society of the United States, Gaithersburg, MD, United States; [5]CAAT, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States; [6]Dow AgroSciences, Indianapolis, IN, United States
mcorvaro@dow.com

Testing of new pesticide active substances includes numerous animal studies with redundancy of evaluated endpoints. Deletion of the 1-year dog test requirement is a clear example of how challenging regulatory change can be. Despite numerous reviews over 20 years, each demonstrating no added value of this test (compared to other studies in the standard database), it remains an unconditional requirement in a few countries. For a global industry, this means that this test will continue to be conducted until there is regulatory agreement across all major markets regarding deletion or waiving of the test requirement. Consideration will be given to the regulatory frameworks of the agrochemical sector, including the potential benefits of an "ICH-like" structure as a vehicle for facilitating improved dialogue and consensus-building among different communities of stakeholders, and greater efficiency and uniformity in the uptake and use of modern approaches to testing and risk assessment.

Session IX-6: 3Rs Communication and Advocacy

Co-Chairs
Kristie Sullivan, Physicians Committee for Responsible Medicine, Washington, DC, United States
Monica Linnenbrink, US EPA, Durham, NC, United States

IX-6-642

Advocating for the replacement and reduction of animals at a global level requires cooperation among diverse stakeholders

Kristie Sullivan
Physicians Committee for Responsible Medicine, Washington, DC, United States
ksullivan@pcrm.org

To advocate more effectively for the replacement and reduction of animals in laboratories, animal protection organizations have built a foundation of scientific and policy experts engaging full time with government, industry, and academia to support and facilitate progress. This includes multi-organization work-sharing at the Organisation for Economic Cooperation and Development (OECD) level, national lobbying, legal instruments, and financial and technical support and training to industry and regulatory scientists. The organization of expert workshops and contributions to the scientific literature representing collaborations between experts from interested stakeholders have been particularly successful. Techniques for using social media targeting different audiences to support policy changes will also be discussed.

IX-6-507

Translating computational toxicology data through stakeholder outreach and communications

Monica Linnenbrink[1], Russell Thomas[2] and Kevin Crofton[2]
[1]US EPA, Durham, NC, United States; [2]National Center for Computational Toxicology, US EPA, Research Triangle Park, United States
linnenbrink.monica@epa.gov

US EPA's National Center for Computational Toxicology (NCCT) develops and uses alternative testing methods to accelerate chemical evaluation, reduce reliance on animal testing, and address the significant lack of chemical toxicity data. Relevant data is generated through high-throughput screening, high-throughput exposure predictions and curating chemistry information. The data is publicly available through the CompTox Dashboard and by using software packages from EPA's website. To increase application of these datasets in chemical safety decisions, EPA uses an array of communications and outreach strategies such as webinars, webpages, fact sheets, media outreach, educational events, training materials, and research collaborations while actively requesting feedback from users as they use the data. This presentation will provide an overview of EPA's implemented communication strategies, discussion about the most and least successful strategies, and future plans for engaging and expanding stakeholder communities.

This abstract does not necessarily reflect U.S. EPA policy

IX-6-91

Pushing for continuous 3Rs improvements: Contributions of a scientific animal welfare organisation

Barney Reed
Research Animals Department, RSPCA, Horsham, United Kingdom
barney.reed@rspca.org.uk

The RSPCA is the world's oldest and largest animal welfare organisation. We work with governments, NGOs and others across 30+ countries in Europe, Asia and Africa to promote animal welfare and help develop regulations. This talk describes our essential role and activities (for more on this, see: https://www.rspca.org.uk/researchanimals) to reduce the conflict between animals and science. Our input is widely respected and our pragmatic liaison with all stakeholders and evidence-based approach allows us to provide constructive challenge and effective advocacy for animals. Examples of how we advance the 3Rs and robust ethical review will be given, e.g. collaborating with learned societies and professional bodies; liaising with government officials; convening scientific meetings and training events; sitting on Animal Welfare and Ethical Review Bodies (similar to IACUCs); visiting research establishments to discuss animal care and use; and producing good practice guidelines. We want to see wider involvement of animal welfare NGOs in these areas worldwide.

IX-6-499

Report on the Unilever and EPA collaboration on developing *in vitro* and *in silico* methods for toxicological risk assessment

Paul Carmichael

Safety & Environmental Assurance Centre, Unilever, Sharnbrook, United Kingdom

paul.carmichael@unilever.com

In July 2015 Unilever and the EPA entered into a three-year cooperative research and development agreement (CRADA) to explore the development of both *in vitro* and *in silico* tools for use in safety assessments. The specific aims of the CRADA were the further development of:

1. ToxCast technologies for the identification of molecular initiating events;
2. High throughput transcriptomics;
3. Integration of metabolic competence into high throughput *in vitro* assays;
4. The translation of results into next generation risk assessments (application of BPAD using reverse dosimetry/IVIVE) using a number of case study chemicals.

This talk will present some of the latest findings and will discuss the great value of multi-stakeholder collaboration for a common purpose. Overall, we hope to illustrate how assessing health risks of chemical ingredients without animal studies can better reflect the actual risk associated with intended human exposure.

IX-6-465

The Human Toxicology Project Consortium: A private-public partnership to promote development and acceptance of pathway-based science

Catherine Willett

The Humane Society of the United States, Gaithersburg, MD, United States

kwillett@humanesociety.org

The Human Toxicology Project Consortium (HTPC) is a coalition of stakeholders from corporate and non-profit communities in the US and EU who share the goal of advancing a mechanistic, biological pathway-based approach to toxicology and disease studies. To accelerate the global implementation of this approach, HTPC focuses on three areas we feel are critical to success: strategic support of the science; communicating the issues to non-scientist stakeholders; and advocating for sustained financial and legislative support. Through a combination of these approaches, we are accelerating development of the necessary scientific solutions, increasing awareness of the importance of this paradigm shift to not only moving away from animal testing, but to improving human and environmental health, and building the foundation for sustained support. In this presentation, I will describe some of the approaches we are taking to achieve this progress.

Reference

https://www.humantoxicologyproject.org

IX-6-329

The impact of animal protection groups on alternatives and animal testing for REACH in the EU

Katy Taylor

Cruelty Free International, London, United Kingdom

katy.taylor@crueltyfreeinternational.org

REACH is the EU regulation on chemical safety. Its implementation has undoubtedly lead to a significant increase in animal testing. However, the legislation has measures within it that should keep this to a minimum. EU animal protection organisation, ECEAE, has been involved in REACH since its implementation. This presentation will give our view on whether REACH has achieved its aims of minimising animal testing and promoting alternatives. It will cover the numbers of animals that have been used and the adoption of new alternative methods. Interesting mechanisms in the legislation such as the testing proposal commenting phase, Board of Appeal cases and updates to REACH guidance, all involve the participation of stakeholders. This presentation will discuss how effective the ECEAE has been at contributing to these areas. In total, the ECEAE believes it has helped avoid testing in tens of thousands of animals, but more can still be done.

IX-6-498

Sharing technology and expertise: A US society promotes the use of alternatives through its membership activities

Erin Hill[1], Kristie Sullivan[2] and David Allen[3]

[1]Institute for In Vitro Sciences, Gaithersburg, MD, United States; [2]Physicians Committee for Responsible Medicine, Washington, DC, United States; [3]Integrated Laboratory Systems, Research Triangle Park, NC, United States

ehill@iivs.org

Differing from many parts of the world, the drive toward non-animal approaches in toxicology in the United States has been prompted more by scientific motivation than legislative mandates. Desiring a forum where non-animal approaches could be explored in more detail, scientists from industry, academic/research institutions and government have helped to grow a scientific society in the US known as the American Society for Cellular and Computational Toxicology (ASCCT). Working through its membership which now totals over 250 scientists, the ASCCT organizes its annual meetings in collaboration with government agencies (e.g. US EPA or FDA), provides a platform to share technological advancements and provides mentorship for young scientists. The creation and growth of ASCCT provides a model for other geographical regions which wish to promote the development and use of alternative methods. In this presentation, an overview of the ASCCT will be combined with responses to a membership survey regarding successes and challenges in implementing non-animal test methods and approaches.

IX-6-82

Survival guide for performing good 3Rs lobbying within the EU policy arena

Francois Busquet
CAAT Europe, Ixelles, Belgium
caat-eu-policy@uni-konstanz.de

With more than 80% of national competences being centralised by the European Commission (EC) on behalf of the EU member states, Brussels is the place to be when it comes to interact with the policy makers (e.g. Members of the European Parliament (MEP)). EC transparency register has collected in March 2017 more than 11,000 entries claiming lobbying activities in the Belgian capital. Animal Welfare and 3Rs are not always a central topic being discussed whereas Brexit, Greek debt, migration crisis, terrorism attacks, glyphosate re-authorization are also steering the attention. In other words, the 3Rs' topic competes with all these major issues that policy makers deal with every day. Therefore, it is essential to focus on few principles in order to better communicate 3Rs and be efficient with policy makers by 1) linking 3Rs with the political agenda, 2) interacting with MEP based on their political color, geographic origin or studies and 3) monitoring MEPs real-time voting using http://www.votewatch.eu/. Moreover, it will be the opportunity to identify some of the roadblocks when it comes to 3Rs when in contact with MEPs.

IX-6-648

A campaign to end invasive chimpanzee research and retire all chimpanzees to sanctuaries: Successful strategies and lessons learned

Kathleen Conlee and Jennifer Ball
Animal Research Issues, The Humane Society of the United States, Washington, DC, United States
kconlee@humanesociety.org

The Humane Society of the United States, in 2006, launched a campaign in the United States to end the use of chimpanzees in invasive research and retire them to appropriate sanctuaries. Since that time, the use of chimpanzees for biomedical research and testing has essentially ended and the number of chimpanzees in laboratories has dropped by approximately 50%, while hundreds more have started making their way to high quality sanctuaries. This presentation will provide an overview of successful strategies that were employed to help reach the goals and lessons learned for the future, including: legal challenges, legislative and policy efforts, scientific reviews of chimpanzee use, financial cost analyses, corporate engagement, public outreach and education and an ongoing effort to build high quality sanctuary capacity in the United States.

Session IX-7: Examining the Gap Between Regulatory Acceptance and Industry Use of Alternative Methods

Co-Chairs
Erin Hill, Institute for In Vitro Sciences, Gaithersburg, MD, United States
Sandra Coecke, European Commission, Joint Research Centre (JRC), Ispra, Italy

IX-7-202

Why regulatory acceptance doesn't necessarily result in industry use of alternatives

Erin Hill and Rodger Curren
Institute for In Vitro Sciences, Gaithersburg, MD, United States
ehill@iivs.org

For many years the Holy Grail of a new alternative test method was development of an OECD Test Guideline resulting in regulatory acceptance and international Mutual Acceptance of Data. However, even after achieving this status, we find that many methods are underutilized. Companies have concerns that reviewers won't accept the data, or that it will take them much longer to make a decision than with animal data. In addition, an international company doesn't want to do two types of tests if one of the countries they sell in requires animal testing. Both situations present higher costs to industry, either in direct costs or in losses due to increased time to market. One solution is removing uncertainty by increased transparency (dialog) between industry and regulators, combined with training of reviewers before and after agency acceptance. The second solution is international harmonization which again requires technical training, dialog and the creation of appropriate infrastructure.

IX-7-154

Good *In Vitro* Method Practices and EU-NETVAL to fill a gap between regulatory acceptance and industry use of alternative methods

Sandra Coecke
European Commission, Joint Research Centre (JRC), Ispra, Italy
sandra.coecke@ec.europa.eu

To ensure regulatory acceptance and industry use of *in vitro* methods good scientific, technical and quality practices should be used in the overall process from development to *in vitro* method implementation. Coordinated by EURL ECVAM, the OECD is developing a Guidance Document on Good *In Vitro* Method Practices (GIVIMP). It targets all key players involved in the process and covers *in vitro* method development, standardisation, validation, harmonisation and international acceptance.

The European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) is a relatively new structure of 37 highly qualified laboratories that has been established (Directive 2010/63/EU) to assist in bringing more resources for the validation of *in vitro* methods. EU-NETVAL has a key role to play in bringing *in vitro* methods from the developer to the *in vitro* method user based on GIVIMP and to implement regulatory accepted *in vitro* methods in their test facilities.

References

Coecke, S. et al. (2005). Guidance on good cell culture practice. A report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim 33*, 261-287.

Coecke, S. et al. (2014). Considerations in the development of in vitro toxicity testing methods intended for regulatory use. In A. Bal-Price and P. Jennings (eds.), *In Vitro Toxicology Systems, Methods in Pharmacology and Toxicology*. New York: Springer.

Coecke, S. et al. (2016). Practical aspects of designing and conducting validation studies involving multi-study trials. *Adv Exp Med Biol 856*, 133-163.

Pamies, D. et al. (2017). Good cell culture practice for stem cells and stem-cell-derived models. *ALTEX 34*, 95-132.

Suter-Dick, L. et al. (2015). Stem cell-derived systems in toxicology assessment. *Stem Cells Dev 24*, 1284-1296.

IX-7-453

A strategy to ensure relevant science for regulatory acceptance and industry adoption

Nicole Kleinstreuer

NICEATM, Research Triangle Park, NC, United States
nicole.kleinstreuer@nih.gov

The development, validation and implementation of new alternative methods is a complex and time-consuming process that requires input from all stakeholders at every stage. Historically, validation has been driven by method developers seeking acceptance, and despite significant resource investments, this has not always translated into regulatory approval or industry adoption. Recent initiatives (e.g. Tox21, Horizon2020, Tissue-Chips) increase biological coverage and mechanistic understanding with testing strategies that are smarter, faster, and more relevant to human health. However, it is difficult for institutional practices to keep pace with scientific advances without a formalized process for coordination, collaboration, and evaluation. The United States has recently launched a national strategy and roadmap for modernizing safety testing, and a key component is the need for constant communication between regulators and industry, with the dual aims of harmonization and utilization.

IX-7-328

Barriers to the uptake of alternatives: An animal protection perspective

Katy Taylor and Laura Rego

Cruelty Free International, London, United Kingdom
katy.taylor@crueltyfreeinternational.org

It is well known that there are both scientific and non-scientific barriers to the adoption of new non-animal methods. This presentation will discuss the barriers from the perspective of animal protection organisations that are closely involved in regulatory testing and provide examples of what we have done to try to overcome them. It will discuss the scientific and bureaucratic barriers at each of the stages towards adoption of an alternative method. These are identified as; development, validation, formal test method adoption, regulatory acceptance and deletion of the animal test method. Using recent examples from EU regulatory sectors and the OECD, evidence is provided for the timescales at each stage and suggestions for how the adoption of alternatives could be speeded up.

IX-7-551

Validation redefined? Validation and regulatory acceptance of alternative methods with an emphasis on test strategies – Results of a joint workshop of BfR and RIVM

Tanja Burgdorf[1], Michael Oelgeschlaeger[1], Jan van Benthem[2], Anne Kienhuis[2], Janine Ezendam[2] and Gilbert Schoenfelder[1]

[1]Experimental Toxicology and ZEBET, Federal Institute for Risk Assessment (BfR), Berlin, Germany; [2]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
tanja.burgdorf@bfr.bund.de

Current validation procedures were developed for individual alternative test methods. These procedures are essential in the process of regulatory acceptance and implementation in legal frameworks for chemical substances. Since a single alternative test method usually cannot replace an *in vivo* test method, computational and *in vitro* tools are combined in testing strategies, covering our mechanistic understanding of the toxicology of interest. There is no procedure how to validate testing strategies and this hinders regulatory acceptance and use. BfR and RIVM organized a multi-stakeholder workshop aimed at defining a strategy to facilitate a more effective process of validation and regulatory acceptance of alternative testing strategies. During the workshop, a mechanism-driven approach for the validation of testing strategies was discussed, as well as drivers and barriers in the process of validation and regulatory acceptance.

Session IX-8: Global Efforts Moving Towards Replacement of Animals

Co-Chairs
Warren Casey, NTP, Research Triangle Park, NC, United States
Herman Koëter, Orange House Partnership, Lucca, Italy

IX-8-739

A new strategic roadmap to establish new approaches for evaluating the safety of chemicals and medical products in the United States

Warren Casey
NTP, Research Triangle Park, NC, United States
warren.casey@nih.gov

Exponential advances in science and technology have not yet resulted in a concomitant increase in our ability to more accurately predict adverse human health effects caused by exposure to chemicals. Achieving the full potential of these advances requires a national strategy for the safe, effective, and timely implementation of human-based, predictive approaches for toxicity testing. Consequently, ICCVAM is coordinating the development of a strategic roadmap for incorporating new approaches into safety testing of chemicals and medical products in the United States that will increase confidence in alternative methods and improve their relevance to human health outcomes while maximizing efficiency and maintaining a commitment the 3Rs. Federal agencies, the regulated community, and international partners will work together to explore new processes for evaluating the safety of chemicals and medical products that will guide the development of new tools, promote the use of flexible and efficient practices to establish confidence in new methods, and facilitate the use of these new approaches by Federal agencies and regulated industries.

IX-8-461

The future of regulatory safety assessment

Herman Koëter
Orange House Partnership, Lucca, Italy
herman.koeter@orangeohouse.eu

In closing WC8 in Rome, 2008 I made the statement that by 2020 the regulatory safety assessment of chemicals will be realized without experimental animal use. Today, we are approaching that date still using animals. However, progress since Rome has been tremendous and technologies such as novel stem cell technologies, organs on a chip and many more developments have found their way to numerous applications. In Spring 2016, the Minister of Agriculture decided to burden the "Netherlands National Committee for the Protection of Animals Used for Scientific Purposes" (NCad in short) with the assignment: "*to develop a roadmap for the phasing out of the use of animals in scientific research*". He further urged the Dutch scientific community to aim at becoming a world leader in animal-free innovations. The lecture addresses the approach of the Netherlands for constructing ambitious yet passable and strategic pathways towards a future of animal free research by recognizing and dealing with the many roadblocks on the path.

Reference

Transition to Non/Animal Research. Netherlands National Committee for the Protection of Animals Used for Scientific Purposes, December 2016, Publication number 201609EN. https://english.ncadierproevenbeleid.nl/

IX-8-691

The 3Rs in action: A Canadian roadmap

Charu Chandrasekera and Phillip Karpowicz

Canadian Centre for Alternatives to Animal Methods, University of Windsor, Windsor, ON, Canada
cpchandr@gmail.com

From the Americas to the Far East, many countries boast alternatives centres, but Canada has lagged behind. Here we present the establishment of the *first* Canadian Centre for Alternatives to Animal Methods (CCAAM), and its subsidiary, the Canadian Centre for the Validation of Alternative Methods (CCVAM) established in collaboration with Canadian regulators. The vision of CCAAM is to promote the replacement of animals in Canadian biomedical research, education, and regulatory testing through 21st century science, innovation, and ethics. CCAAM will serve as a leader and nexus for alternatives to animal testing in Canada by collaborating with academia, industry, non-profit, and government scientists as well as ethicists, policy makers, and the public to develop, validate, and promote ways to reduce and replace animal testing through extensive Research, Academic, and Regulatory initiatives. Here we unveil our plans to contribute to global alternatives efforts in a uniquely Canadian way.

IX-8-530

Towards a 21st century roadmap for biomedical research and drug discovery

Troy Seidle[1], Catherine Willett[2] and Lindsay Marshall[3]

[1]Research & Toxicology, Humane Society International, Montréal, QC, Canada; [2]Animal Research Issues, Humane Society of the United States, Gaithersburg, MD, United States; [3]Humane Society International, London, United Kingdom
tseidle@hsi.org

Despite investment of billions of dollars, development of new drugs remains elusive and immensely expensive, mainly due to insufficient efficacy and/or unacceptable toxicity in humans. There is growing recognition that a stronger focus on human-relevant pre-clinical data is needed. Similar limitations in chemical safety assessment has led to significant investment and development of more relevant, efficient methods to understand chemical toxicity. These new approaches focus on biological pathways or networks (Adverse Outcome Pathways or AOPs). Recent investments in medicine and disease research have led to several similar projects related to systems biology. Health research and toxicology would benefit greatly from coordination of existing efforts in both fields. Toward this aim, we have held international workshops bringing together scientists and representatives from funders and regulatory agencies to identify major issues and discuss the path forward. Here we present conclusions and recommendations from this series of events.

IX-8-487

A roadmap towards animal-free regulatory safety testing – Results of a workshop on transition to non-animal research

Anne Kienhuis, Jacqueline van Engelen, Lianne de Wit and Xandra Gravestein

RIVM, Bilthoven, The Netherlands
anne.kienhuis@rivm.nl

The Netherlands National Committee for the protection of animals used for scientific purposes (NCad) has, in its 2016 report "Transition to non-animal research", presented the transition objective for the complete phasing out of animal procedures in the field of regulatory safety assessment by 2025. RIVM takes the responsibility to draw up a roadmap towards animal-free regulatory safety testing, and organizes a national multi-stakeholder workshop in June 2017. A key principle for the discussion during the workshop is to maintain safety, however, based on animal-free methods and strategies only. A main challenge for the national ambition to succeed is to extend it to all levels of organization in the international field of regulatory testing. The results of the national workshop will be presented. The audience is invited to discuss key aspects necessary to move forward towards animal-free testing for safety assessment.

IX-8-485

Scientific conference on non-animal approaches – The way forward

Katrin Schutte and Susanna Louhimies

DG Environment, European Commission, Brussels, Belgium
katrin.schutte@ec.europa.eu

The European Commission (EC) organised a scientific conference in 2016, engaging the scientific community in a debate on how to exploit scientific advances to develop scientifically valid non-animal approaches (alternatives to animal testing).

Attendees from many scientific disciplines and sectors with expertise in animal or non-animal approaches explored in panel discussions benefits and limitations of different models, how to improve the quality/predictivity of them, and how to overcome roadblocks hampering substantial 3R progress. Participants discussed recommendations for the research community, funding bodies, scientific journals, the EC and stakeholders on:

– validity of animal models in different sectors
– state of play and way(s) forward for non-animal approaches in different areas of research and testing
– promising technologies for reducing and replacing animals in R&D
– best practice to advance research integrity, funding and reporting

The conference was announced in the EC Communication responding to the European Citizens Initiative "Stop Vivisection" and aimed to contribute to the goal of ultimately phasing out animal testing.

Reference

http://ec.europa.eu/environment/chemicals/lab_animals/3r/scientific_conference_non_animal_approaches_en.htm

Theme IX: Global Cooperation

Poster Presentations

IX-103

Enabling alternatives to animal testing in science based regulation: Enhancing prediction of carcinogenic potential of agrochemicals, an EPAA-led project

Matthias Vey[1], Susanne Belz[2], Jonathan Crozier[3], Charles Laroche[3], Irene Manou[3], Annette Mehling[4] and Renate Weissenhorn[3,5]

[1]IFRA, Brussels, Belgium; [2]European Commission DG JRC, Ispra, Italy; [3]EPAA, Belgium; [4]BASF, Ludwigshafen, Germany; [5]European Commission DG GROW, Brussels, Belgium
mvey@ifraorg.org

The European Partnership for Alternative Approaches to Animal Testing (EPAA) is a unique platform, involving stakeholders from the European Commission, European trade associations, and companies from 8 industry sectors, with the declared goal to jointly foster the acceptance of the "3Rs" by facilitating the knowledge exchange between the stakeholders to meet regulatory requirements through better and more predictive science.

The mission of EPAA revolves around two major axes: 1) to promote the implementation of alternative approaches, and 2) to enhance the acceptance, harmonization and mutual recognition of tests by regulators at national, European and international levels. This is achieved by a number of means including conferences, publications and projects. Just as one example, a new project *Enhancing prediction of carcinogenic potential of agrochemicals* is expected to contribute to a reduction of carcinogenicity studies in the crop protection sector. The projects rely heavily on the commitment of the stakeholders. This presentation aims at showcasing the work done by EPAA as well the new and ongoing scientific projects.

References

European Commission (2005). The 3Rs Declaration. https://circabc.europa.eu/sd/d/3a0533fa-cfbf-4536-a7ca-f9c7c10f3eae/3rs-declaration.pdf

Sistare, F. D. et al. (2011). An analysis of pharmaceutical experience with decades of rat carcinogenicity testing: Support for a proposal to modify current regulatory guidelines. *Toxicol Pathol 39*, 716-744. doi:10.1177/0192623311406935

Van der Laan J. W. et al. (2016). Prediction of the carcinogenic potential of human pharmaceuticals using repeated dose toxicity data and their pharmacological properties. *Frontiers in Medicine 3*, 45. doi:10.3389/fmed.2016.00045

IX-104

Development of *in vitro* transactivation assay to detect human androgen receptor agonist/antagonist using 22Rv1/MMTV_GR-KO cells

HeeSeok Lee[1], YongSun Song[1], Jin-Hyang Suh[1], Min-Ki Shin[1], Yoon Jae Cho[1], Hyun-Suk Oh[1], Myung-Sil Hwang[1], Guiim Moon[1], Yooheon Park[2], Jin-Hwan Hong[1] and Yong Eui Koo[1]

[1]Food Safety Risk Assessment Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Chungcheongbuk-do, South Korea; [2]Research Institute of Biotechnology & Medical Converged Science, Dongguk University, Seoul, South Korea
hslee0515@gmail.com

Endocrine disrupting chemicals are exogenous molecules that can be interfered the action of sexual hormones including androgen receptor. Cooperation between the international bodies working on the test guidelines is managed by the Endocrine Disruptors Testing and Assessment (EDTA) task force and the Validation Management Group for Non Animal Testing (VMG-NA) in OECD. We have previously reported that 22Rv1 cells, a human prostate cancer cells contained functional Androgen Receptor (AR), might be an appropriate model for the evaluation of potential androgenic compounds. In this study, we established the test protocol and optimized the testing condition for AR TA assay using glucocorticoid receptor (GR) knock out (KO) 22Rv1/MMTV cells. In conclusion, 22Rv1/MMTV_GR-KO AR TA assay might be a quick and relatively inexpensive method, which can be used to screen large numbers of chemicals for their potential to activate or inhibit AR-mediated gene transcription.

IX-114

Screening on AR agonistic and antagonistic effects of pesticides by OECD *in vitro* assays

HeeSeok Lee, Da-Woon Jung, Gyeong-Yong Oh, Hyun-Suk Oh, Myung-Sil Hwang, Guiim Moon, Jin-Hwan Hong and Yong Eui Koo

Food Safety Risk Assessment Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Chungcheongbuk-do, South Korea
hslee0515@gmail.com

OECD has provided a standardized method to search the endocrine disrupting chemicals. Various results were reported that several pesticides can be shown adverse effect in low concentration exposure via

their unidentified-endocrine disrupting effects. In this study, we compared two *in vivo* screening systems using AR-EcoScreen™ (OECD TG458) and 22Rv1/MMTV_GR-KO (OECD project 4.99) cell lines for detection of AR agonistic and antagonistic effects through testing of 74 pesticides. Among the tested chemicals, 33 pesticides have been found to AR antagonistic effect in two AR TA assays. On the other hand, 16 pesticides were exhibited AR antagonistic effect differently between AR-EcoScreen™ AR TA and 22Rv1/MMTV_GR-KO AR TA because of their different intrinsic toxicity against each applied cell line. These results revealed that it provides valuable information about AR agonist/antagonist effects on various pesticides by OECD test guideline and me-too test method.

IX-121

Dialogue is crucial: The German National Committee as an effective harmonization instrument for more animal welfare

Justyna Chmielewska[1], Bettina Bert[1], Barbara Grune[1], Andreas Hensel[1] and Gilbert Schönfelder[1,2]

[1]Federal Institute for Risk Assessment, Berlin, Germany;
[2]Charité-Universitätsmedizin Berlin, Berlin, Germany
Justyna.Chmielewska@bfr.bund.de

The implementation of the Dir. 2010/63/EU requires not only adequate legal measures on the national level, but also cooperation of all parties directly involved in animal research and the authorization process. A harmonized interpretation of the legal provisions in every Member State and throughout the European Union is essential to ensure animal welfare and to provide legal security for the research.

German National Committee regards fostering a culture of dialog and achieving a harmonized approach as one of its key tasks. An interdisciplinary approach based on close cooperation of experts from different fields (biomedical and veterinary science, law, ethics etc.) and the competent authorities makes the work particularly effective. Ensuring legal compliance and implementation of the newest scientific findings in laboratory animal science in practice are the highest priority. The outcome of the past 3 years will be presented including examples.

References

Bert et al. (2016). Considerations for a European animal welfare standard to evaluate adverse phenotypes in teleost fish. *EMBO J 35*, 1151-1154.

Bert et al. (2016). The animal experimentation quandary: Stuck between legislation and scientific freedom. *EMBO Rep 17*, 790-792.

Grune et al. (2014). Animal welfare: Rules for assessing pain in lab animals. *Nature 512*, 28.

IX-128

Transition to non-animal research. About the possibilities for phasing out animal procedures and stimulating innovation without laboratory animals

Marjolein Schilders-van Boxel, Henriëtte Bout, Jan-Bas Prins, Herman Koëter, Coenraad Hendriksen, Wim de Leeuw, Reineke Hameleers, Pieter Roelfsema and Leane van Weereld

Ministry of Economic Affairs, Netherlands National Committee for the protection of animals used for scientific purposes (NCad), The Hague, The Netherlands
Marjolein.vanboxel@rvo.nl

World leader in innovations without laboratory animals by 2025. That is the aim of the Dutch Minister for Agriculture, Martijn van Dam. In March 2016, the Minister asked the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) to draw up a schedule for the phasing out of animal procedures. In December 2016, the NCad presented its advisory report "Transition to non-animal research – *About the possibilities for phasing out animal procedures and stimulating innovation without laboratory animals*".

Although there is scientific, economic and social potential for innovations without laboratory animals, according to the NCad, these are currently not being sufficiently exploited to promote and accelerate the transition process. Only with a broad-ranging and coordinated effort by the ministries involved and other stakeholders can significant progress be made in reducing the use of laboratory animals in research. In its report the NCad makes recommendations under three different themes: Clear transition objectives, Transition strategy and Management of the transition.

If we are to make the transition to non-animal research methods, we must make a paradigm shift away from existing mindsets and practices. That way, says the NCad, we can focus heavily on innovations without laboratory animals in a number of fields in the period up to 2025.

In the case of *regulatory research*, the NCad sees potential for a significant reduction in the use of laboratory animals. The use of laboratory animals in regulatory safety testing of chemicals, food ingredients, pesticides and (veterinary) medicines can be phased out by 2025, whilst maintaining the existing safety level. The same applies to the use of laboratory animals for the release of biological products, such as vaccines. At this stage, regulatory pre-clinical research cannot be phased out at the same pace.

In the field of *fundamental scientific* research, the opportunities for a substantial reduction in the use of laboratory animals vary from one field to another. The NCad recommendation to the Minister for Agriculture concerns the development of a ten-year vision for each area of fundamental scientific research (or for each cluster of disciplines) in consultation with the public and the scientific community. These visions must include clear transition objectives that are linked to the core focus of the area of research concerned. They must also give an insight into the potential of innovations without laboratory animals in these areas.

The NCad believes that, in the field of *applied and translational research*, more rapid progress can be made than is being made at the present time. There is a great deal of innovative potential that could be better exploited. In this context, the NCad advises the Minister for Ag-

riculture to focus more heavily on innovations without laboratory animals, amongst others in the field of the development of human models for human diseases and by promoting cross-sectoral and multidisciplinary collaboration on innovation policy. That way, the Netherlands can be an international leader in the field of innovations without laboratory animals in this area of research by 2025.

The transition to non-animal research methods will not happen on its own; it will require management and focus. International collaboration involving all stakeholders is the key to success. The NCad advises the Minister for Agriculture to play a guiding role in the process, and to also involve other ministries in order to ensure that a consistent and coherent policy is developed at national level. In addition, the NCad recommends establishing an Agenda for Innovation Without Laboratory Animals, based on a joint approach by all national stakeholders. This Agenda must focus on specific objectives that are both ambitious and achievable.

This poster will give an overview of the advisory report by the NCad and the actions taken by the stakeholders ever since.

Reference

https://www.ncadierproevenbeleid.nl/documenten/rapport/2016/12/15/ncad-opinion-transition-to-non-animal-research

IX-166

German web-based solution AnimalTestInfo for publishing non-technical project summaries

Justyna Chmielewska[1], Bettina Bert[1], Julia Vietze[1], Daniel Butzke[1], Antje Dörendahl[1], Nora Leich[1], Barbara Grune[1], Andreas Hensel[1] and Gilbert Schönfelder[1,2]

[1]Federal Institute for Risk Assessment, Berlin, Germany; [2]Charité-Universitätsmedizin Berlin, Berlin, Germany
Justyna.Chmielewska@bfr.bund.de

In Germany, the Federal Institute for Risk Assessment (BfR) is responsible for the publication of the non-technical project summaries according to the Directive 2010/63/EU. A web-based solution has been created to facilitate the workflow between applicants, competent authorities and the BfR, and to ensure an easy accessibility for the interested public (https://www.animaltestinfo.de). It allows users a search-term based retrieval providing a comfortable tool to search for the purpose of experiments, application of the 3R, animal species or any other type of information provided in the NTS. This user-friendly approach opens new channels to make data on animal experiments fully accessible and transparent. Moreover, the database offers a great opportunity to analyze the data provided by the NTS and to detect those fields of animal testing where research for alternative methods is urgently required. The database enhances transparency and contributes to promoting animal welfare in the future.

Reference

Schönfelder, G. (2015). Laboratory animals: German initiative opens up animal data. *Nature 519*, 33.

IX-211

Use of alternative methods to assess skin irritation of chemical mixtures

Amy Sheppard, Ellen Baker, Dieldrich Bermudez, James Blattner, Kevin Boyd, Stefanie O'Neal, Trannum Rahim and Edward Whittle

Human Safety, SC Johnson, Racine, WI, United States
AMSheppa@scj.com

SC Johnson (SCJ) has a long history of using alternative methods to reduce dependency on animals in toxicity testing. An analysis of data using OECD methods has resulted in SCJ's development of a testing scheme to help support skin classifications of chemical mixtures. Data from the Bovine Corneal Opacity and Permeability (BCOP; OECD 437), EpiDerm™ Skin Corrosivity Test (SCT; OECD 431) and the Corrositex® assay (OECD 435) were compared with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification and/or existing *in vivo* data. Titratable acid/alkaline reserve (TAR) was taken into account for mixtures with an extreme pH. Results show that the use of these assays together with a weight of evidence approach can support skin classifications for chemical mixtures, and avoid a potential overclassification based solely on the GHS manual calculation method.

References

OECD Test Guideline 437 (2009). Bovine Corenal Opacity and permeability Test Method for Identifying Ocular Corrosives and Severe Irritants.

OECD Test Guideline 431 (2014). In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method.

OECD Test Guideline 435 (2015). In Vitro Membrane Barrier Test Method for Skin Corrosion.

Young, J. R. et al. (1988). Classification as corrosive or irritant to skin of preparations containing acidic or alkaline substances, without testing on animals. *Toxico In Vitro 2*, 19-26.

IX-225

Assessment of China EpiSkin™ skin corrosion and irritation tests Integrated Testing Strategies

Nan Li[1], Yanfeng Liu[1], Lizao Chen[1], Nathalie Alepee[2], José Cotovio[2] and Alice Cai[1]

[1]L'Oréal R&I China, Shanghai, People's Republic of China; [2]L'Oréal Research & Innovation, Aulnay sous Bois, France
nli@rd.loreal.com

Recently, appeal of *in vitro* alternative methods has been rising in toxicological research as well as in cosmetic industry in China. In response, China EpiSkin™ skin corrosion and irritation test methods were developed, according to OECD TG 431 and TG 439, respectively. The present study aimed at evaluating the use of the China EpiSkin™ as an Integrated Approach to Testing and Assessment (IATA), adopted by OECD in 2015 as GD 203. These texts provide guidance on the integration of existing and new information in a modular approach for classification and labelling.

Both bottom-up and top-down testing strategies were applied to a set of 60 substances representing various chemicals classes of different physical states. Results demonstrated that the integration into both strategies reached a high overall accuracy of 90% for sub-categorization. This study could bring a future extension of application and a possible implementation of alternative testing strategy in China.

IX-355

Disseminating OECD TG 491 and OECD TG 492 tests in Brazil

Talita Marin[1], Rodrigo Silva[1], Marize Valadares[2], Estela Cruvinel[3], Carlos Santos[4], Vanessa Rocha[5], Alisson Cardoso[1] and Eduardo Pagani[1]

[1]LNBio/CNPEM, RENAMA, Campinas, Brazil; [2]LFTC, UFG, Goiania, Brazil; [3]PluriCell Biotech, São Paulo, Brazil; [4]INTERTOX, São Paulo, Brazil; [5]Natura Inovação e Tecnologia de Produtos, Cajamar, Brazil
talita.marin@lnbio.cnpem.br

This project aims at providing proficiency and disseminating the ocular toxicity tests OECD TG 491 and TG 492 in Brazil. It is sponsored by the Brazilian Federal Government (CNPq) on behalf of RENAMA, the Brazilian Network on Alternative Methods. It involves five institutions: one private non-profit research center (LNBio); one academic research center (Federal University of Goiás); one contract research organization (Intertox); one cell line manufacturer (PluriCell Technologies) and one cosmetic manufacturer (Natura). These institutions with different backgrounds and located more than 1,000 Km apart are connected by an Enterprise Laboratory Platform – ELP composed of a Laboratory Information Management Systems – LIMS and Electronic Laboratory Notebook – ELN. This allows remote and secure access to project data, responsibility assignment and activities monitoring by all organizations involved. This consortium's model provides strong integration and delivers better results and might be replicated by others nations.

References

OECD Test No. 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

OECD Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage.

IX-358

Cross-sector initiatives to reduce, refine and replace regulatory vertebrate ecotoxicity tests

Nikki Gellatly and Natalie Burden

NC3Rs, London, United Kingdom
nikki.gellatly@nc3rs.org.uk

Ecotoxicity testing is undertaken to assess the impact of chemicals on wildlife populations and ecosystems. Fish, birds and amphibians are the most commonly used vertebrate species in regulatory environmental safety assessment. This area uses a large number of animals annually, but has historically received less attention in terms of applying the 3Rs than the mammalian toxicity testing undertaken to assess human safety. The UK's National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) began a dedicated programme of work in the area of ecotoxicology in 2008, supported by a working group of experts in the field across academia, government agencies (including regulators), contract research organisations, and industry (international agrochemicals, consumer goods, and petrochemicals companies). This presentation will highlight three current cross-sector initiatives being undertaken by the NC3Rs-led working group to apply the 3Rs in regulatory ecotoxicology.

IX-426

Serious eye damage/eye irritation assessment: Reliable and relevant implementation of SkinEthic™ HCE reconstructed human corneal test method in Asia Pacific region

Virginie Leblanc[1], Mariko Yokota[2], Marie Helene Grandidier[1], Yuki Sato[2], Daisuke Yoshida[2], Daiki Kyoutani[3], José Cotovio[1] and Nathalie Alépée[1]

[1]L'Oréal Research & Innovation, Aulnay sous Bois, France; [2]Cosmos Technical Center, Tokyo, Japan; [3]Nikoderm Research, Osaka, Japan
vleblanc@rd.loreal.com

Assessment of ocular irritation risk is an international regulatory requirement. To demonstrate the worldwide applicability of the SkinEthic™ Human Corneal Epithelium (HCE) Eye Irritation Test (EIT) validated test method for chemical categorization, the reliability in Asia Pacific region was assessed in Japan (Cosmos Technical Center). In addition, the possibility of an extended shipping/storage time (e.g., > 4 days), with no impact on the performance of the test method was explored.

After extended tissues transit, there liability assessed on 40 chemicals showed a within and between reproducibility greater than 95%. After extended tissues storage, the relevance evaluated on 119 chemicals showed a 86.5% accuracy, 96.1% sensitivity and 69% specificity.

Thus, performances of SkinEthic™ HCE EIT test method after extended shipment as well as storage remain in agreement with regulatory validation criteria endorsing its integration as a Validated Reference Method in the OECD Test Guideline 492.

IX-475

A survey of global acute systemic toxicity test requirements to support a push towards harmonized acceptance of alternative strategies

Esther Haugabrooks and Kristie Sullivan

Physicians Committee for Responsible Medicine, Washington, DC, United States
ehaugabrooks@pcrm.org

Ongoing efforts to reduce and replace *in vivo* tests for alternative acute systemic toxicity testing in the United States must consider global testing requirements due to the global marketplace in which most companies participate. Evolving test requirements challenge companies to maintain regulatory compliance and full replacement of specific tests will require harmonized acceptance of test methods and strategies. The objective of this presentation is to provide an overview of the current global acute toxicity regulations and propose approaches that can be created or implemented to reduce and replace the use of animals in regulatory toxicology. We cataloged acute systemic toxicity requirements for a variety of sectors by gathering information in online resources and surveying regulatory agencies and chemical companies. This information will help to shape strategies to replace *in vivo* acute systemic toxicity tests in the US and speed harmonization of reduction and replacement strategies.

IX-538

Toward research and *in vitro* testing service to replace the animal testing of cosmetics in China

Yao Qin, Shujun Cheng, Yu Chen and Yihui Ke

Chinese Center for Alternative & Evaluation, Guangzhou CHN-ALT Biotech Co. Ltd, Guangzhou, People's Republic of China
chnalt@163.com

The *in vitro* science laboratory of Chn-Alt, since 2010, specializing in technical services for cosmetics *in vivo* biology testing, as well as R&D toxicology-predictive *in vivo* methods on human healthy and ecology. Through the integration of *in silico* analyses, *in vivo* testing, diverse types of modern genomics and multi-organ on chip, their efforts and mission are to fit well into the 1R replace principle and 21st toxicology concepts. As a first founded independent third-part institute in China, Chn-Alt *in vivo* science not only covered cosmetics toxicology assessment including skin irritation, skin sensitization, ocular irritation and genetic toxicology, but also covered *in vivo* efficacy testing including anti-aging, anti-oxidant and skin biology related assessment.

Excitedly, at presently, the Chinese Center for Alternative & Evaluation that is sponsored by Chn-Alt has held seven times "Chinese conference of alternative methods", which now is the biggest and the most important academic meeting for pushing *in vivo* science development in China. The 3Rs concepts and more ethical ways to conduct science research and industries application are popular accepted in China. There is a big obstacle for regulations accepted alternatives in China, but with the industries and testing agencies to applied these *in vivo* methods, this process will be shorter and easier.

IX-575

Implementation of Reconstructed Human Epidermis (RHE) in Brazil

Rodrigo De Vecchi[1], Vanja Dakic[1], Guilherme Mattos[1], Anne-Sophie Rigaudeau[2], Nathalie Alépée[3], Cristina Garcia[1], Charbel Bouez[4] and José Cotovio[3]

[1]L'Oréal Research & Innovation, Rio de Janeiro, Brazil; [2]Episkin, Lyon, France; [3]L'Oréal Research & Innovation, Aulnay sous Bois, France; [4]L'Oréal Research & Innovation, Clark, NJ, United States
rvecchi@rd.loreal.com

Skin corrosion/irritation data are essential for safety and efficacy evaluation of topically applied products and chemicals. In Europe, the full ban of animal testing was implemented in 2013 and validated methods using Reconstructed Human Epidermis (RHE) replace the use of animals for chemicals classification. In Brazil, a bill was implemented in 2014 aiming the ban on animal testing for cosmetics in 2019, and strengthening the need of robust alternative models. This work describes the first validated RHE implementation in Brazil, SkinEthic™ RHE model, evaluating different functional and histological parameters. Results are consistent confirming the robustness of the model and same quality as the original one validated by ECVAM and OECD. More than 20 chemicals were tested and correctly classified for skin irritation/corrosion using tissues produced in Brazil. The highly reproducible results confirm the quality and robustness of SkinEthic™ RHE model and methods.

IX-584

A "bottom-up approach" to accelerate new opportunities in alternatives and Adverse Outcome Pathway (AOP) practice in China

Tina Qu[1] and Catherine Willett[2]

[1]Chinese Center for Alternatives Research and Evaluation, GuangZhou, People's Republic of China; [2]The Humane Society of the United States, Gaithersburg, MD, United States
tqu@ccare.net.cn

Since the 3Rs were introduced in China in the 1990s, constant work finally resulted in adoption of the 3R policy by the Ministry of Science and Technology in 2006. Alternatives have gradually received attention via a "bottom-up" approach: a combination of academic and industrial promotion and technology training, which will be discussed. Two national academic societies on alternatives were established in 2014. Established in 2015, a Chinese AOP working team will begin public training in 2017. Workshops by the Guangdong Inspection and Quarantine Bureau have trained more than 1,200 people in alternatives. Standardization work has resulted in 20 OECD TGs adopted by the Ministry of the Environment and one by the China Food and Drug Administration. The future implementation of alternatives is expected to accelerate under several important new national policies, such as the Plan for the improvement of consumer product standard and quality, Made in China 2025, and the Belt and Road initiatives.

IX-670

Advantages of establishing a national Animal Welfare Body Platform; Experiences from the Netherlands

Harry Blom

Utrecht University, Utrecht, The Netherlands
h.j.m.blom@uu.nl

EU Directive 2010/63 on the protection of animals used for scientific purposes requires that breeder, supplier and user establishments shall set up an animal welfare body and appoint a designated veterinarian who acting as an advisory member. The animal welfare body has several mandatory advisory and monitoring tasks regarding animal welfare, 3Rs and a variety of internal processes. In addition, the animal welfare body is frequently charged with duties like the monitoring of adequate education, training and skills of all levels of personnel and establishing an internal auditing programme.

In 2016 a national platform for animal welfare bodies was established in the Netherlands to allow for the exchange of experiences and to share information. At present animal welfare bodies of breeders, industry, academia, CRO's and a variety of small organisations have joined. The national platform aims to install work groups, produce advisory reports and codes of practice and to organize meetings and workshops. The presentation will provide an overview of recent developments within the Netherlands as well as on attempts to initiate international collaboration.

IX-763

Prediction of cholestasis by integrating multiple *in vitro* measurements in a systems framework

M. K. Narasimha and Kalyanasundaram Subramanian
Syngene International Ltd., Bangalore, India
Narasimha.Mandyam@syngeneintl.com

Cholestatic liver injury is one of the most severe manifestations of drug induced liver disease (DILD), This along with mixed forms of injury accounts for almost 50% of reported hepatic drug toxicity. Cholestatic liver injury produced by drugs may lead to unnecessary diagnostic and therapeutic interventions and increased injury severity if one doesn't understand the agent that caused the injury to begin with. While cholestatic injury results from dysfunction of bile formation and disposition, there are multiple mechanisms that could all lead to this end state. From a drug-development perspective, it becomes important to understand if a compound affects these mechanisms, the degree of effect and the impact of these effects together. An understanding of the affected mechanisms also allows chemists to design out the features of a compound that lead to the effect.

To aid in this, we have created a systems model that integrates multiple *in vivo* inputs and assesses the combined effect of multiple mechanisms on the liver. In the past, the model to predict cholestasis considered the effect of a compound on uptake, efflux and reflux transporters and its modulation by changes in mitochondrial oxidative phosphorylation (Subramanian et al., 2008). However, more recently we have added a simple model of the cytoskeletal network, the ROCK/MLCK pathway, its impact on canalicular contractility and consequently, on the secretion of bile acids into the canaliculus. We will show how this model was built and validated using data generated from the HepaRG cell system (BioPredic International). While Burbank et al. (2017) used time-lapse fluorescent video microscopy to observe the canalicular dynamics and radio-labelled TCA to measure the bile-acid clearance, we show that we can use simple biochemical measurements to predict what was observed in these experiments. Furthermore, we will demonstrate that for certain drugs no individual measurement is sufficient and that we need to analyse multiple measurements in an integrated manner to get the true picture of the cholestatic impact of a drug on the liver.

References

Burbank, M. G., Sharanek, A., Burban, A. et al. (2017). From the Cover: MechanisticInsights in Cytotoxic and Cholestatic Potential of the Endothelial Receptor Antagonists Using HepaRG Cells. *Toxicol Sci 157*, 451-464. doi:10.1093/toxsci/kfx062

Subramanian, K., Raghavan, S., Rajan Bhat, A. et al. (2008). A systems biology based integrative framework to enhance the predictivity of in vitro methods for drug-induced liver injury. *Expert Opin Drug Saf 7*, 647-662.

Late Breaking Abstracts

1000

Induced pluripotent stem cell-derived hepatocyte-like cells could offer a personalizable human cell source for *in vitro* safety and efficacy studies

Kelly R. Stevens

Departments of Bioengineering and Pathology, University of Washington, Seattle, WA, United States

ksteve@uw.edu

Induced pluripotent stem cell-derived hepatocyte-like cells could offer a personalizable human cell source for *in vitro* safety and efficacy studies. However, differentiation of iPSCs towards hepatocyte-like cells (iPSC-Heps) currently results in cells with an immature hepatic phenotype that most closely resembles fetal, not adult, hepatocytes. For example, iHeps persistently express the fetal marker alpha fetoprotein (AFP) and also lack activity of detoxification enzymes such as CYP2A6 and CYP3A4. We hypothesize that controlled presentation of cues found in the normal liver microenvironment (e.g., cell-cell interactions, cell-matrix interactions, and vascular perfusion) will improve iHep maturation. Here, I will summarize our work in building novel biofabrication platforms to recapitulate aspects of liver structure and perfusion *in vitro*. Our preliminary data using these platforms demonstrates the importance of both cell-cell interactions and perfusion on iPSC-Hep phenotype and function.

1001

PREPARE guidelines for planning animal research and testing

Adrian Smith[1], Eddie Clutton[2], Elliot Lilley[3], Kristine Hansen[4] and Trond Brattelid[5]

[1]Norecopa, Oslo, Norway; [2]Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom; [3]RSPCA, United Kingdom; [4]Norwegian University of Life Sciences, Oslo, Norway; [5]Western Norway University of Applied Sciences, Bergen, Norway

adrian.smith@norecopa.no

Scientists and the animal welfare movement are concerned about the poor reproducibility and translatability of many animal experiments. Our experience indicates there are many factors to be considered beyond those topics listed in reporting guidelines.

We have produced a set of planning guidelines for scientists and facilities, called PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence). PREPARE covers all stages of quality assurance, from facility management to individual procedures. PREPARE is presented alongside a 2-page checklist and a website with hundreds of links to specific guidelines on each topic.

More information on https://norecopa.no/PREPARE

1002

Generation and formatting of Affimer® affinity binders for the inhibition of the PD-L1/PD-1 pathway

Chris Miller[1], Emma Jenkins[1], Estelle Adam[1], Anna Ah-San Tang[2], Andrew Wilcox[1], Christina Rauber[1], Lindsay Mcmorran[1], Matt Johnson[1] and Amrik Basran[1]

[1]Avacta Life Sciences, Wetherby, United Kingdom; [2]University of Leeds, Leeds, United Kingdom

chris.miller@avacta.com

Affimer reagents and therapeutics are a class of non-antibody binding proteins that have been engineered for a wide range of applications where antibodies and aptamers have limitations. They are produced without the use of animals, can be used to detect difficult targets, can easily be formatted for a wide range of applications and can be easily and cost effectively manufactured. Using phage display, we have identified competitive binders to a range of targets, including the immune check point, Programmed death-ligand 1 (PD-L1). PD-L1 plays an important role in immune homeostasis and blockade of the PD-L1/PD-1 pathway using antibodies has demonstrated impressive anti-tumour responses in cancer patients. Our inhibitors have been shown to be highly selective for PD-L1 with KD's of single digit nM as determined by BIAcore. We have shown that the scaffold is amenable to being engineered to make multimers (dimers, trimers and tetramers) as well as being formatted to extend the serum half-life.

1003

Pre-competitive collaboration: Data sharing for effective progression

Laura Johnston

Lhasa Limited, Leeds, United Kingdom
laura.johnston@lhasalimited.org

Pre-competitive collaboration is becoming increasingly common across industry as organisations strive to work together for mutual gain. Collaborating through data sharing initiatives can occur through an increasing number of flexible approaches, most commonly operating through an 'honest broker'1, whereby an independent, third party is responsible for coordinating the activities of a consortium.

This poster aims to explore a number of pre-competitive data sharing initiative case studies, demonstrating benefits through: working together to influence the introduction of changes to regulation; reducing the need to duplicate experiments; quantifiably improving *in silico* predictions and models; and, therefore, ultimately making more informed decisions. Such collaborative efforts are particularly pertinent on account of the economic and social pressure to replace, reduce and refine the use of animal tests for the safety assessment of chemicals across a wide range of industries.

Reference

Elder, D. P., White, A., Harvey, J. et al. (2015). Mutagenic Impurities: Precompetitive/Competitive Collaborative and Data Sharing Initiatives. *Org Process Res Dev 19*, 1486-1494. doi:10.1021/acs.oprd.5b00128

1004

Accelerating transition to 21st century toxicology: *Hydra* emerges as a model organism for ecotoxicity testing of bulk and nanomaterials

Mohammad Abdulkader Akbarsha[1], Murugadas Anbazhagan[2], Zeeshan Mohammed[2], Thamaraiselvi Kaliannan[2], Babu Rajendran Ramaswamy[2] and Franz P. Gruber[3]

[1]Mahatma Gandhi-Doerenkamp Center, Bharathidasan University, Tiruchirappalli, India; [2]Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, India; [3]Doerenkamp Zbinden Foundation, Kuesnacht, Switzerland
akbarbdu@gmail.com

Tox-21c and REACH legislation emphasize the use of invertebrate model organisms for testing of chemical entities. In this context, we examined the suitability of *Hydra*, a freshwater cnidarian, for risk assessment of environmental chemicals. Though simple in organization and biology, it is much complex compared to cultured cells, which make it an amenable organism for ecotoxicity testing. We took to advantage the whole genome sequencing of *Hydra* which revealed conserved sequences and signaling pathways. The poster will present data on the effect of nano-copper and its bulk form on morphology, feeding, regeneration, and growth rate. Molecular end points such as DNA damage, apoptosis, cell cycle arrest, and transcriptional regulation of antioxidant genes were also investigated. TEM analysis revealed sub-cellular alterations and accumulation of nanoparticles within the cells of *Hydra*. The data substantiate the use of *Hydra* as a convenient model organism for ecotoxicity testing.

1005

Investigating a novel mechanism of developmental neurotoxicity: The impact of quaternary ammonium compounds on neurodevelopment through modulation of lipid homeostasis

Josi Herron[1], Kelly Hines[2], Hideaki Tomita[2] and Libin Xu[1,2]

[1]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States; [2]Department of Medicinal Chemistry, University of Washington, Seattle, WA, United States
jh0618@uw.edu

Environmental chemicals have been implicated in the etiology of neurodevelopmental disorders; however, there is a lack of information regarding the developmental neurotoxicity (DNT) hazard of environmental chemicals and limited knowledge on DNT mechanisms. Thus, we proposed a novel mechanism that environmental toxicants impact neurodevelopment through disruption of lipid homeostasis, as lipids are essential for neurodevelopment. Our previous studies revealed a class of disinfectants, quaternary ammonium compounds (QACs), that disrupt sterol homeostasis similar to biochemical changes in Smith-Lemli-Opitz syndrome, a neurodevelopmental disorder. We hypothesized that QACs could impact neurodevelopment through modulation of lipid homeostasis. We found that *in utero* exposure to an environmentally relevant mixture of QACs altered sterol homeostasis in the PND0 brain. Moreover, individual QACs reduced neural precursor cell survival *in vitro*, which could be detrimental to neurogenesis.

1006

Evaluation of Skin Allergy Risk Assessment (SARA) Integrated Approach to Testing and Assessment (IATA) using six ingredients and two product types

Carl Westmoreland, Nora Aptula, Maria Baltazar, James Butcher, Richard Cubberley, Nicola Gilmour, Cameron Mackay, Gavin Maxwell, Ruth Pendlington, Joe Reynolds and Evita Vandenbossche

SEAC, Unilever, Bedford, United Kingdom
carl.westmoreland@unilever.com

Our aim is to apply mechanistic and clinical understanding to develop a risk assessment approach for skin allergy that: doesn't require new animal test data; addresses novel exposure scenarios; better characterises our uncertainty.

The Skin Allergy Risk Assessment Integrated Approach to Testing and Assessment (SARA IATA) is a tiered approach that predicts the probability of human skin sensitisation occurring following a given product exposure, with explicit uncertainty using two defined approaches (DA): a probabilistic, weight of evidence (WoE) clinical potency model designed to use existing *in vivo*, *in vitro* or *in silico* hazard information to inform an initial prediction of skin allergy risk followed by a skin toxicokinetic (TK) model-based tier that can further reduce uncertainty in the risk prediction, as required.

The SARA IATA has been evaluated using six case study ingredients and two products types: results from this analysis will be shown alongside conclusions and next steps.

1007

Nortis microfluidic organ-on-chip technology: Human tissue microenvironments for basic research, drug toxicology and efficacy testing

Henning Mann

Research & Development, Nortis, Inc., Woodinville, WA, United States

mann@nortisbio.com

Nortis has developed a technology that is used to recapitulate functional units of human organs in microfluidic cell culture devices (chips). Such organ models include vasculature, kidney, and liver models for toxicology studies, blood-brain barrier models for drug transport studies, and vascularized tumor/tissue microenvironments for drug efficacy studies. These models exhibit *in-vivo* like barrier function, transporter polarity, and metabolic and enzymatic activity. Common architecture to these models is a small, continuously perfused tubular structure of endothelial or epithelial cells that is surrounded by a 3D extracellular matrix based on collagen I. Co-culture of cells can be compartmentalized by embedding cells into the extracellular matrix. By applying growth factor gradients, endothelial microvessels can be coaxed into sprouting vascular networks that interact with cells embedded into the matrix, thus building vascularized microenvironments. The Nortis tissue models are designed as alternatives to animal testing ranging from academic and pharmaceutical research to toxicology studies in the cosmetic industry.

References

Chang, S. Y. Weber, E. J., Ness, K. V. et al. (2016). Liver and Kidney on Chips: Microphysiological Models to Understand Transporter Function. *Clin Pharmacol Ther 100*, 464-478. doi:10.1002/cpt.436

Chang, S. Y., Voellinger, J. L., Van Ness, K. P. et al. (2017). Characterization of rat or human hepatocytes cultured in microphysiological systems (MPS) to identify hepatotoxicity. *Toxicol In Vitro 40*, 170-183.

Kelly, E. J., Wang, Z., Voellinger, J. L. et al. (2013). Innovations in preclinical biology: ex vivo engineering of a human kidney tissue microperfusion system. *Stem Cell Res Ther 4 Suppl 1,* S17.

Tourovskaia, A., Fauver, M., Kramer, G. et al. (2014). Tissue-engineered microenvironment systems for modeling human vasculature. *Exp Biol Med 239*, 1264-71.

Weber, E. J., Chapron, A., Chapron, B. D. et al. (2016). Development of a microphysiological model of human kidney proximal tubule function. *Kidney Int 90*, 627-37.

1008

Not quite there: BASF's experience in replacing the acute toxicity tests for agrochemical formulations

Susanne Kolle[1], Andrew Van Cott[2], Charles Hastings[2], Bennard Van Ravenzwaay[1] and Robert Landsiedel[1]

[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany; [2]BASF Corporation, Research Triangle Park, NC, United States

susanne.kolle@basf.com

Acute toxicity testing is routinely conducted on agrochemical formulations as a regulatory requirement. Several non-animal methods have gained regulatory acceptance but their predictive capacity for agrochemical formulations is usually unknown.

Comparing *in vivo* and *in vitro* skin irritation and corrosion data indicates a lack of applicability of the current protocol of the *in vitro* skin irritation test (OECD 439) for agrochemical formulations.

None of the evaluated protocols (HetCam, BCOP (OECD 437), two modified BCOP protocols, ICE (OECD 438), EpiOcular™ ET-50) was sufficiently sensitive to predict UNGHS Cat 1 agrochemical formulations correctly. The EpiOcular™ EIT (OECD 492) was predictive for non-irritants and agrochemical formulations have been taken up in the OECD TG.

The GHS additivity approach for acute oral, dermal and respiratory toxicity used for formulations containing at least one toxic ingredient falsely characterized the hazards of formulations with interacting ingredients.

References

Kolle, S. N., Rey Moreno, M. C., Mayer, W. et al. (2015). The EpiOcular™ Eye Irritation Test is the Method of Choice for the In Vitro Eye Irritation Testing of Agrochemical Formulations: Correlation Analysis of EpiOcular Eye Irritation Test and BCOP Test Data According to the UN GHS, US EPA and Brazil ANVISA Classification Schemes. *Altern Lab Anim. 43*, 181-98.

Kolle, S. N., Van Cott, A., van Ravenzwaay, B. and Landsiedel R. (2017). Lacking applicability of in vitro eye irritation methods to identify seriously eye irritating agrochemical formulations: Results of bovine cornea opacity and permeability assay, isolated chicken eye test and the EpiOcular™ ET-50 method to classify according to UN GHS. *Regul Toxicol Pharmacol 85*, 33-47.

Kolle, S. N., van Ravenzwaay, B. and Landsiedel, R. (2017). Regulatory accepted but out of domain: In vitro skin corrosion and irritation tests for agrochemical formulations. *Regul Toxicol Pharmacol*, in press.

Schrage, A., Gamer, A. O., van Ravenzwaay, B. and Landsiedel, R. (2010). Experience with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations. *Altern Lab Anim 38*, 39-52.

van Cott, A., Kolle, S. N., Hastings, C. and Landsiedel, R. (2017) GHS additivity formula: (Non-) Applicability to agrochemical formulations that contain interacting ingredients. Manuscript in preparation.

1009

Comparative analysis of the difference of toxicity test between Chinese and European Pharmacopoeia

Maoling Wei[1], Bo Liu[2] and Xin Mu[3]

[1]West China Hospital, Sichuan University, Chengdu, People's Republic of China; [2]Chengdu Rongsheng Pharmaceutical Co., Ltd, Chengu, People's Republic of China; [3]Chengdu, People's Republic of China

maolingwei@hotmail.com

Background: The legislative changing is one of the key roles to promote the practice on animal alternatives in biomedical research. A study also showed that the alternative animal researches were insufficient and suggested more studies on legislation, policy leading, medical education, etc.

Objective: To understand the current status and compare the differences on animal used requirements between the China and European pharmacopoeia and in biomedical research.

Methods: Two authors independently reviewed The European Pharmacopoeia 8th edition issued in 2013 and The Pharmacopoeia of the People's Republic of China 10th issued in 2015 and analyzed the requirements on new medicine and biomedical products on animal use requirements, which including first testing, the second testing and results classification, etc. If any differences about the judgment were solved by discussion. The main findings were summarized in a descriptive way as follows.

Results: a) There is a similar testing methods and process required for animal used in drug testing and biological products to detect toxicity test in China and European pharmacopoeia.

b) The toxicity test for medicines and biomedical products were similar in first animal testing.

c) The main differences between the two pharmacopoeias were: The preparation of sample, the top dose of injection on biological products in European Pharmacopoeia, etc. The number of animal used in the second testing double than its first testing in China. The results of the tested animal observation time longer in China than its in Europe. The animal's blank control was also required for biomedical products in China pharmacopoeia, while no requirement in Europe pharmacopoeia.

Conclusion: The requirements for animal use for toxicity test between Chinese and European Pharmacopoeia were similar. However, there are some differences on the products testing; animals use details and observation time between the two pharmacopoeias. It seems more consideration of science and human benefit while increase the number of animal in China. It is necessary to better balance on the legislative, scientific and ethical of animal use in biomedical research in future.

References

The Pharmacopoeia of the People's Republic of China. China Medical Science and Technology Press. 2015.

The European Pharmacopoeia, 8th edition. Available at http://pharmeuropa.edqm.eu

Wei, M. (2016). Study on the Current Status of Medical Alternative Animal Researches (In Chinese). *Chinese Medical Ethics 29*, 304-307.

1010

Lifestage-specific organotypic modeling platform for adverse outcome pathways of male reproductive and developmental processes

Rachel Shaffer, Carly Wilder, Catherine Ryu, Hannah Yi, Sun Young Park, Sung Woo Hong, Collin White, Tomomi Workman, William Griffith and Elaine Faustman

Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States

rms14@uw.edu

Early life development of the male reproductive system is vulnerable to disruption from chemical exposure, particularly during critical windows of susceptibility. We have established a novel 3D mouse testicular co-culture *in vitro* system to evaluate the effects of exposures during postnatal days (PND) 9-25. This time period has been identified as a critical window in the mouse testis through transcriptomic analysis of publically available data and a developmental timeline based on a literature search. We analyzed long-term viability, testosterone production and morphology up to 16 days in culture to characterize baseline features of the system. Sertoli, Leydig and spermatogonial germ cells were identified using cell-type specific markers of proliferation and differentiation with Western blots and immunofluorescence. There was a biphasic pattern of testosterone concentration in culture, which is expected during this period because of the transition from fetal to adult Leydig cell populations. A known testicular toxicant, cadmium, was used to evaluate effects of chemical exposure during this critical window of susceptibility. We exposed the co-culture system to cadmium (2.5, 5 and 10 μM concentrations) on days *in vitro* (DIV) 2, 6, and 15 and characterized testosterone production, cytotoxicity, cell viability, and protein expression after 24 hours of exposure. Initial studies have observed dose dependent cytotoxicity and differential susceptibility based on time of exposure. These lifestage-specific quantitative results can be interpreted within the context of an Adverse Outcome Pathway (AOP) and demonstrate the potential of our model to capture adverse outcomes in proliferation, steroid regulation and spermatogenesis pathways of male reproductive development.

1013

The Lush Prize Session: Adverse outcome pathways – Will they deliver a superior alternative to animal testing?

Rebecca Ram

Scientific consultant, Lush Prize, Flitwick, United Kingdom
rebecca@lushprize.org

Animal testing is resource-intensive in terms of money, time and animal use, posing scientific and ethical challenges. It is also well known to be highly fallible. Consequently it has become impossible to evaluate fully all the chemicals being developed or already on the market. Research to replace animals with *in vitro, in chemico* and *in silico* technologies has achieved considerable success to date.

However, faster progress could be made with a transparent and systematic framework that provides an underlying rationale to develop and assess human-relevant methods and reliably interpret their data output. The Adverse Outcome Pathway (AOP) concept offers such a framework and, with it, the possibility of creating a genuinely robust toxicology and risk assessment strategy that is fit for purpose.

Since 2012, the Lush Prize has awarded annual bursaries in science, training, lobbying , public awareness as well as awarding several young researchers each year, to encourage the replacement of animals in safety testing and research and a shift in recognition of human-relevant toxicity pathways and 21st century science. The AOP framework remains a key focus of the Lush Prize and 2015 saw the first Black Box Prize awarded to several organisations, for their successful development of test methods corresponding to the AOP for skin sensitisation.

Attendees at WC10 are invited to the Lush Prize session, to hear an overview of the prize and the AOP framework and to participate in a discussion/Q&A with experts in the field, on the progress of adverse outcome pathways to date.

1016

A novel hTERT immortalized human podocyte cell line modelling the glomerular filtration barrier

Mattias Wieser, Teresa Francisci, Susanne Neubert, Johannes Grillari and Regina Grillari-Voglauer

Evercyte GmbH, Vienna, Austria
matthias.wieser@evercyte.com

Kidney podocytes are key components of the glomerular filtration units forming mechanical epithelial barriers that retain high molecular weight factors like albumin, while letting pass lower molecular compounds like glucose. Thus, they are instrumental in generating the primary urine filtrate and subsequently associated with severe proteinuria based kidney diseases when lost or damaged induced by drugs or toxicants. Due to limited availability of standardizable and relevant *in vitro* models for simulation this essential glomerular filtration barrier, we have focused on the development of hTERT immortalized human podocytes that maintain functions and features of the corresponding normal cell counterparts. The resulting cell line PODO/TERT256 is characterized by continuous proliferation and morphologically resembles human primary podocytes. Synaptopodin, nephrin, synaptopodin and WT-1, considered as podocyte markers, were observed by immunofluroscence microscopy. The cells are sensitive to puromycin aminonucleoside (PAN) treatment when differentiated in 2D culture. Moreover, the cells can be cultured as 3D model in combination with microvascular endothelial cells (HDMVEC/TERT164). This model retains albumin as intact barrier and shows leakage upon PAN treatment, which can be partly rescued by mizoribine. In summary, these data indicate that the PODO/TERT256 cell line mimics features of human primary podocytes and might therefore represent an easy and fast to handle model system for podocyte injury and the development of kidney disease.

1017

Long-term exposure of immortalized keratinocytes to arsenic induces EMT, impairs differentiation in organotypic skin models and mimics aspects of human skin derangements

Regina Weinmüllner[1], Michael Mildner[2], Johannes Grillari[3] and Petra Heffeter[4]

[1]Department of Biotechnology, CD Laboratory on Biotechnology of Skin Aging, BOKU, Vienna, Austria; [2]Department of Skin Biology, Medical University of Vienna, Vienna, Austria; [3]Evercyte GmbH, Vienna, Austria; [4]Cancer Research Institute, Medical University of Vienna, Vienna, Austria

regina.weinmuellner@boku.ac.at

Arsenic is one of the most important human carcinogens and environmental pollutants. However, the evaluation of the underlying carcinogenic mechanisms is challenging due to the lack of suitable *in vivo* and *in vitro* models, as distinct interspecies differences in arsenic metabolism exist. Thus, it is of high interest to develop new experimental models of arsenic-induced skin tumorigenesis in humans. Consequently, aim of this study was to establish an advanced 3D model for the investigation of arsenic-induce skin derangements, namely skin equivalents, built from immortalized human keratinocytes (NHEK/SVTERT3-5). In contrast to spontaneously immortalized HACAT cells, NHEK/SVTERT3-5 cells more closely resembled the differentiation pattern of primary keratinocytes. With regard to arsenic, our results showed that while our new cell model was widely unaffected by short-time treatment (72 h) with low, non-toxic doses of ATO (0.05–0.25 μM), chronic exposure (6 months) resulted in distinct changes of several cell characteristics. Thus, we observed an increase in the G2 fraction of the cell cycle accompanied by increased nucleus size and uneven tubulin distribution. Moreover, cells showed strong signs of de-differentiation and upregulation of several epithelial-to-mesenchymal transition markers. In line with these effects, chronic contact to arsenic resulted in impaired skin-forming capacities as well as localization of ki67-positive (proliferating) cells at the upper layers of the epidermis; a condition termed Bowen's disease. Finally, chronically arsenic-exposed cells were characterized by an increased tumorigenicity in SCID mice. Taken together, our study presents a new model system for the investigation of mechanisms underlying the tumor promoting effects of chronic arsenic exposure.

Author Index

ACKNOWLEDGEMENTS

The Congress Co-Chairs and the Alternatives Congress Trust would like to express their gratitude to all institutions that are sponsoring the 10th World Congress on Alternatives and Animal Use in the Life Sciences (WC10).

In addition we are indebted to all colleagues serving on the Scientific Committee, the Theme Coordinators, the Program Management Team and to the staff of our congress organizer FASEB, since without their continuous support we would not have been able to create an attractive scientific and social WC10 program.

Platinum Sponsors

L'ORÉAL Research & Innovation

P&G

ACT ALTERNATIVES CONGRESS TRUST

Unilever

Silver Sponsors

THE HUMANE SOCIETY OF THE UNITED STATES

HUMANE SOCIETY INTERNATIONAL

HUMAN TOXICOLOGY PROJECT CONSORTIUM
Advancing a New Paradigm for Assessing Chemical Safety

Johnson & Johnson

Bronze Sponsors

Kao Corporation

Kanebo COSMETICS INC.

ALTERNATIVES RESEARCH & DEVELOPMENT FOUNDATION

charles river | every step of the way.

Congress Notepads and Pens Sponsor

epaa
The European Partnership for Alternative Approaches to Animal Testing

Congress Badges/Lanyard and Online App Sponsor

charles river | every step of the way.

Supporters

SOT | Society of Toxicology
Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

BASF
We create chemistry

syngenta

ifra
The International Fragrance Association

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SANOFI

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

CAAT

DOERENKAMP-ZBINDEN-STIFTUNG · DOERENKAMP-ZBINDEN-FOUNDATION

COLGATE-PALMOLIVE COMPANY

ExxonMobil

DEPARTMENT OF ENVIRONMENTAL & OCCUPATIONAL HEALTH SCIENCES
UNIVERSITY of WASHINGTON · SCHOOL OF PUBLIC HEALTH

Institute for Risk Analysis & Risk Communication
Strengthening the foundations of risk assessment

The University of Washington EDGE Center
Exposures, Diseases, Genomics & Environment

ALTEX Proceedings

http://www.altex-edition.org
http://altweb.jhsph.edu/altex

Vol. 6, No. 1 (2017)

Issued by
Society ALTEX Edition, Kuesnacht, Switzerland

ALTEX Edition Editorial Office Europe
Sonja von Aulock (Editor in chief)
Franz P. Gruber (CEO)
Petra Mayr (Editor TIERethik)
Carolin Rauter (Technical editor)
Goran Krummenacher (Webmaster)

Address
Society ALTEX Edition
Weinmanngasse 86
8700 Kuesnacht ZH, Switzerland
Phone: +41 44 380 0830
Fax: +41 44 380 0832
e-mail: editor@altex.ch

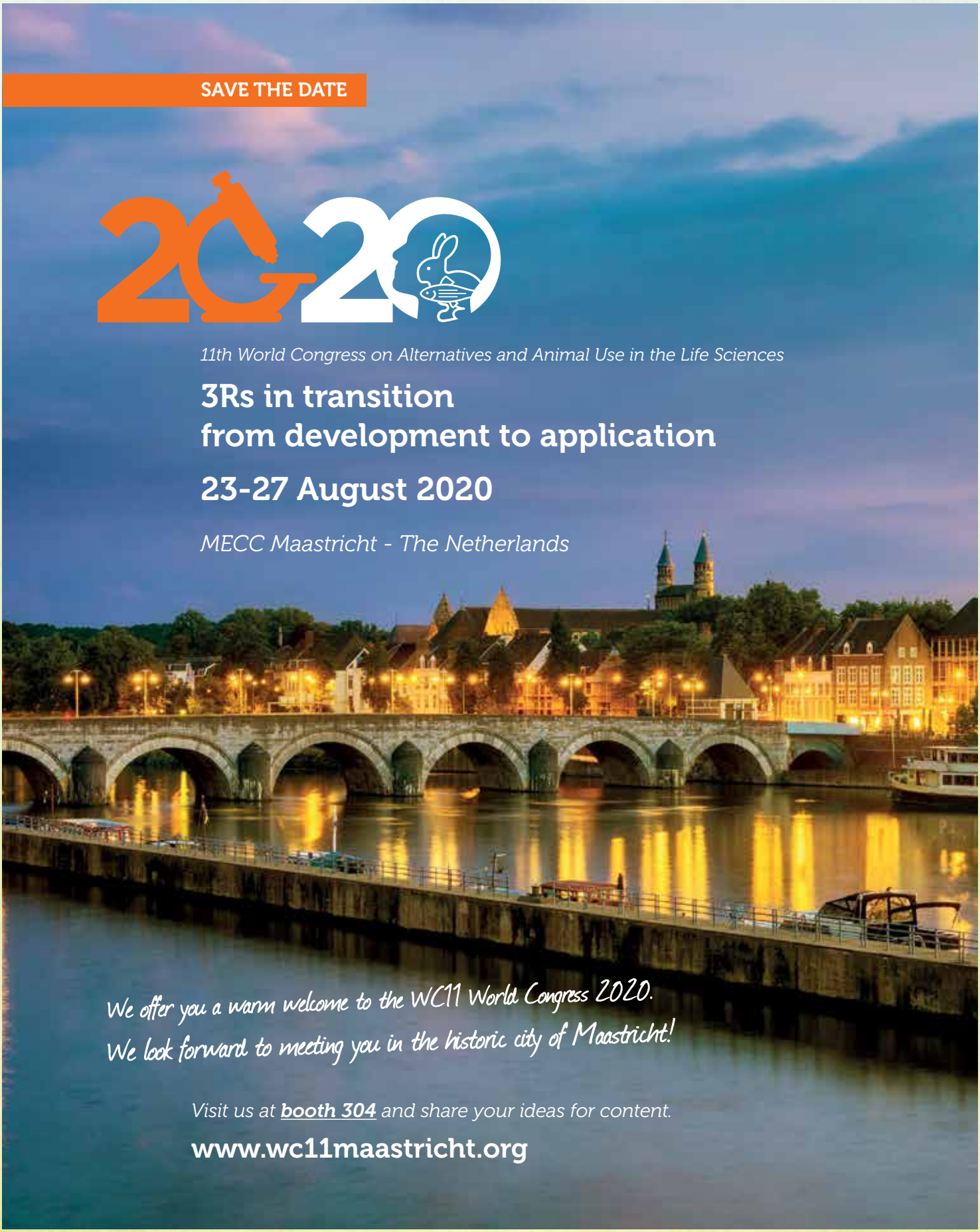
ALTEX Edition Editorial Office USA
Joanne Zurlo (North American Editor)
Thomas Hartung
Michael Hughes

Address
Johns Hopkins University Baltimore
615 N Wolfe Street, W7032 Baltimore
MD 21020, USA
Phone: +1 443 287 2515
Fax: +1 410 614 2871
e-mail: jzurlo@jhsph.edu

Layout
H. P. Hoesli

ALTEX Proceedings are published online:
www.altex-edition.org
www.altweb.jhsph.edu/altex

ALTEX Proceedings publishes Abstract Books and Proceedings of scientific conferences and symposia on the development and promotion of alternatives to animal experiments according to the 3R concept of Russell and Burch: Replace, Reduce, and Refine in cooperation with the organizers of the respective meeting.


SAVE THE DATE
2020
11th World Congress on Alternatives and Animal Use in the Life Sciences
3Rs in transition
from development to application
23-27 August 2020
MECC Maastricht - The Netherlands
We offer you a warm welcome to the WC11 World Congress 2020.
We look forward to meeting you in the historic city of Maastricht!
Visit us at booth 304 and share your ideas for content.
www.wc11maastricht.org

TT21C.org

Safety Science in the 21st Century

Establishing global scientific partnerships to develop and apply next generation research, tools and thinking for mechanistically-based (non-animal) risk assessments: assuring the future safety of our consumers, workers and the environment.

Visit TT21C.org to learn more about our work and to access details of Unilever's presentations at the 10th World Congress on Alternatives and Animal Use in the Life Sciences, including:

- Paul Carmichael: Predictive safety approaches following ten years of TT21C: From inspiration to application
- Paul Carmichael: Report on Unilever and EPA collaboration on developing *in vitro* and *in silico* methods for toxicological risk assessment
- Carl Westmoreland: Cellular stress, tipping points and application in risk assessment: An international research programme.
- Andy White: Developing systems for safety decision making: Challenges for improving confidence

Introduction to the Society of Toxicological Alternatives and Translational Toxicology (TATT), China

- **The Society of Toxicological Alternatives and Translational Toxicology (TATT), under the Chinese Society of Toxicology (CSOT) was founded in October, 2014**
- **TATT unites hundreds of pioneer scientists and regulators working on alternatives from academic institutions, universities, companies, and governmental agencies in China as well as international scientists**

Prof. Shuangqing Peng, Ph.D, Chair of TATT, Vice-President of CSOT

Aims and Responsibility of TATT

Promote the development, validation, regulatory acceptance, international coordination, and application of non-animal alternatives in toxicological research and risk assessment

Provide a platform for national and international scientific communications on toxicological alternatives and translational toxicology, and strong collaborations between different parties

Facilitate professional education and dissemination of non-animal alternatives and translational toxicology, promote the cultivation and growth of young scientists and experts

Annual meeting of TATT and scientific communications

Opening ceremony of the 2nd TATT meeting

Delegates at the 2nd TATT meeting, 2016

Poster exhibition at the 2nd TATT meeting

Since the foundation of TATT, International Conferences on Toxicity Testing Alternatives and Translational Toxicology have been held yearly. Over 500 national and international delegates now participate in these meetings (2017, Nanjing; 2016, Hangzhou; 2015, Xi'an).

Expertise and research collaboration on alternatives

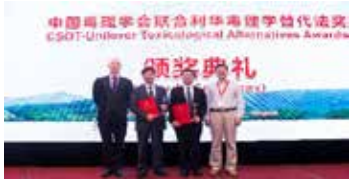
Research collaboration between AMMS and Unilever

Visiting the UK NC3Rs 2013

Chinese TT21C/AOP roadmap workshop, 2015

TATT has established broad collaborations worldwide with scientists and research institutes, universities, regulatory agencies, and industrial companies, to promote the developmental, regulatory acceptance, and application of alternatives in China.

Professional awards and scientific publications



CSOT-Unilever Toxicological Alternatives Awards

Outstanding Young Investigator Award, 2016

Publications on alternatives and TT21C/AOP

Many important awards have been issued by TATT such as CSOT-Unilever Excellent Contribution Award and CSOT-Unilever Innovative Award on Toxicological Alternatives, and Outstanding Young Investigator Award. Each year hundreds of papers and books on alternatives are published in China.

2018 (4th) International Conferences on Toxicity Testing Alternatives and Translational Toxicology (Annual meeting of TATT, 2018)
& The 2nd Asian Congress 2018 on Alternatives and Animal Use in the Life Sciences (Guangzhou, China; October, 2018)

Eliminating Research Involving Animals – Our Contributions to the 10th World Congress Alternatives and Animal Use in the Life Sciences

Oral Presentations

- *Use of transcriptional profiling in in vitro systems to determine the biological activity of chemicals of interest,* Naciff J.
- *Industry approaches for botanical safety evaluation, without the use of animals;* Daston G, Vandermolen K, Naciff J, Mahony C.
- *Round Table: Future of Big Data in 3Rs and Recommendations;* Mahony C (Moderator), Currie R, Daston G, Kleinstruer N, vandeWater B.
- *Safety assessment of topical ingredients- a case study;* Taalman R, Alépée N, Ashikaga T, Clouet E, Cluzel M, Del Bufalo A, Goebel C, Hoffmann S, Kern P, Klaric M, Kuehnl J, Mewes K, Miyazawa M, Petersohn D, Van Vliet E, Gilmoure N, Hibatallah J, Hutchison L.
- *Cosmetics Europe Analysis of the Robustness of Testing Strategies for UN GHS Classification for Serious Eye Damage/Eye Irritation of Chemicals*; Alépée N, Adriaens E, Bagley D, Desprez B, Hibatallah J, Mewes K, Pfannenbecker U, Sala À, McNamee P.
- *Cosmetics Europe Eye Programme: Relevance to Integrated Approaches on Testing and Assessment for Serious Eye Damage/Eye Irritation;* McNamee P, Alépée N, Adriaens E, Bagley D, Desprez B, Hibatallah J, Mewes K, Pfannenbecker U, Sala À.
- *Development of a Repeated Dose Toxicity Mode of Action – Based Ontology;* Birk B, Blaauboer B, Boobis A, Carmichael P, Cronin M, Curie R, Daston G, Desprez B, Jennings P, Klaric M, Kroese D, Mahony C, Ouédraogo G, Piersma A, Richarz A, Schwarz M, van Benthem J, van de Water B, Vinken M.
- *Exploring the value of new approach methodologies in read-across: the parabens as a collaborative EU ToxRisk - Cosmetics Europe case study;* Ouedraogo G, Van der Burg B, Mahony C, Naciff J, Ellison C, Detroyer A, Bury A, Drewe W, Long T, Kamp7, Dinant Kroese H, Escher S, Cull T, White A, Dent M, Blaauboer B, Keller D, Willighagen E, Cronin M, Currie R, Gräpel R, Van de Water B, Mombelli E.

Poster Presentations

- *Validation and application of the 3D human reconstructed skin micronucleus assay (RSMN) using the EpiDermTM tissue to the safety assessment of cosmetics ingredients;* Pfuhler S, Aardema, M, Roy S, Scheirer J, Kulkarni R, Mun G, Wilt N, Costin E, Curren R, Barnett B, Hoffmann S, Hewitt NJ, Desprez B.
- *Use of multiparametric in vitro mode of action approaches for genetic toxicity assessment;* Pfuhler S, Allemang A, DeAbrew N , Downs T , Shan Y.
- *3D Skin Comet assay: Genotoxicity assessment addressing the dermal route of exposure;* Hoffmann S, Kerstin Reisinger K , Brinkmann J, Down T, Fischer A, Haase A, Henkler F, Liebsch M, Luch A, Petrick C,, Pirow R, Reus A, Said A , Schäfer-Korting M, Schulz M, Pfuhler S.
- *Lessons from Read-Across Case Studies for Repeated-Dose Toxicity;* Mahony C, Schultz TW, Cronin MTD.

Poster Presentations

- *Use of newly validated 3D reconstructed human skin genotoxicity assays and hen's egg test micronucleus in testing strategies improve predictions in safety assessments of cosmetic ingredients*; Fautz R, Desprez B, Hoffmann S, Hewitt NJ, Fowler P, Reisinger K, Kuehnl J., Ouédraogo G; Kenny J, Pfuhler S.
- *Testing Strategies for UN GHS Classification for Serious Eye Damage/Eye Irritation of Chemicals: Cosmetics Europe Analysis;* Alépée N, Adriaens E, Bagley D, Desprez B, Hibatallah J, Mewes K, Pfannenbecker U, Sala À, McNamee P.
- *The importance of understanding physico-chemical properties of chemicals in the evaluation of serious eye damage/eye irritation: Cosmetic Europe analysis.* Desprez B, Adriaens E, Alépée N, Bagley D, Hibatallah J, Mewes KR, Pfannenbecker U, Sala À, McNamee P.
- *Cosmetics Europe Eye Programme: Relevance to Integrated Approaches on Testing and Assessment for Eye Hazard;* McNamee P, Alépée N, Adriaens E, Bagley D, Desprez B, Hibatallah J, Mewes K, Pfannenbecker U, Sala À.
- *Use of the Porcine Corneal Opacity and Reversibility Assay (PorCORA) for testing detergent and cleaning products identified as in vivo Category 1 (Cat. 1) due to persistence of ocular tissue damage according to the UN Globally Harmonized System (GHS) of classification;* Eskes C, Bertein C, Carathers M, DeGeorge G, Hermann M, Jones P, McNamee P, Varsho B.
- *Assessing the allergenic potential of proteins from their sequence and 3D structure using novel computational approaches;* Gerberick GF, Gunalan V, Limviphuvadh V, Ryan C , Kern P, Maurer-Stroh S.
- *Better understanding of bioavailability of cosmetic ingredients: Results from Cosmetics Europe skin bioavailability project;* Schepky A, Cubberley R, Duplan H, Eilstein J, Ellison C, Grégoire S, Hewitt N, Jacques-Jamin C, Lange D, Roe A, Rothe H, Klaric M.
- *Bridging the gap from external to internal exposure: use of in silico, PBPK and in vitro methods to predict the biokinetics of topically applied cosmetic ingredients;* Klaric M, Cubberley R, Duplan H, Eilstein J, Ellison C, Grégoire S, Hewitt N, Jacques-Jamin C, Lange D, Roe A, Rothe H, Schepky A.
- *Cosmetics Europe Assessment of Non-Animal Approaches for Predicting Skin Sensitization;* Klaric M, Alépée N, Allen D, Ashikaga T, Casey W, Clouet E, Cluzel M, Del Bufalo A, Gellatly N, Goebel C, Hoffmann S, Kern P, Kleinstreuer N, Kuehnl J, Mewes K, Miyazawa M, Petersohn D, Strickland J, Van Vliet E, Zang D.
- *An in vitro strategy for informing relative potency of chemicals acting through a common mode of action – Pyrrolizidine alkaloids;* Mahony C, Blackburn K, Lester C, Pfuhler S, Troutman J.

P&G as Presenters and/or (Co-)Authors underlined

Please visit our experts George Daston, Frank G Gerberick, Catherine Mahony, Pauline McNamee, Jorge Naciff, Stefan Pfuhler & Harald Schlatter at our booth (number 106) or visit us @ http://us.pg.com/sustainability/community-impact/policies-practices/animal-welfare-alternatives

Platinum Sponsor

ADVANCING SCIENCE **WITHOUT SUFFERING**

BIOMED 21

- ENGAGING SCIENTISTS & FUNDING BODIES IN **STRATEGIC DIALOGUE**
- FUNDING 21ST CENTURY **RESEARCH ROADMAPS** FOR HUMAN DISEASE
  hsi.org/rfp

TOWARD A **HUMAN-SPECIFIC PARADIGM** FOR MEDICAL RESEARCH

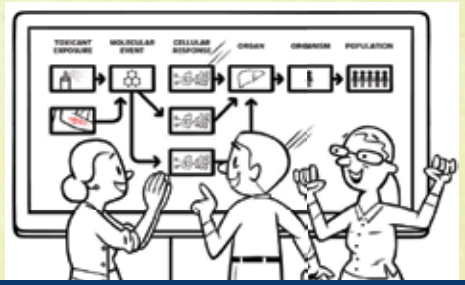
DRIVING GLOBAL REGULATORY ALIGNMENT, ACCEPTANCE & USE OF ALTERNATIVE APPROACHES ACROSS INDUSTRY SECTORS

SUPPORTING NON-ANIMAL METHOD TRAININGS & INFRASTRUCTURE DEVELOPMENT IN EMERGING ECONOMIES

21ST CENTURY TOXICOLOGY
OUTREACH & TRAINING

- AOP HISTORY & OVERVIEW COURSE
- AOPs 101/201 LECTURE SERIES
- NON-TECHNICAL VIDEO SERIES

humantoxicologyproject.org
aopwiki.org/training/aops



HUMANE SOCIETY INTERNATIONAL

THE HUMANE SOCIETY OF THE UNITED STATES

HUMAN TOXICOLOGY PROJECT **CONSORTIUM**

YOUR ANIMAL WELFARE AND 3RS ACCOMPLISHMENTS JUST GREW BY 1,000 PERCENT.

THE NORTH AMERICAN 3Rs COLLABORATIVE

Advancing Science, Innovation, and Welfare Through Sharing

Introducing **The North American 3Rs Collaborative**, a passionate community of professionals who have come together to share ideas and further 3Rs awareness, current technologies, and approaches. Our mission is to advance the education and science of the 3Rs – refining, reducing, and replacing the use of animals in research. **Visit us in booth 402** to learn more about our mission, resources, and membership details or find out more at **www.NA3RsC.org**.

Subscribe to ALTEX

Support open access publication of 3Rs research

SUBSCRIPTION SERVICE

ALTEX Edition, Weinmanngasse 86
8700 Kuesnacht ZH, Switzerland
Phone: +41 44 380 0830, Fax: +41 44 380 0832
e-mail: subs@altex.ch

First name

Last name

Institute/Library
(if applicable)

Address

State

Zip code

Country

e-mail

Date/signature

Please send completed form to the above address.

ALTEX (four issues):

☐ **Individual subscription**
$ 120 / 102 €

☐ **Library**
$ 240 / 204 €
(companies, institutes, libraries)

☐ **Reduced**
$ 62 / 55 €
(students, animal protection organizations, selected scientific societies)

Prices include postage for all countries.

The subscription is automatically renewed unless it is cancelled by the end of the year.

I want to pay by

☐ credit card ☐ check

☐ electronic bank transfer ☐ please send me an invoice

ALTEX is available online:
http://altweb.jhsph.edu/altex and www.altex-edition.org