Meeting Report

Summer School on Innovative Approaches in Science

1 Introduction

"The most dangerous phrase in science is: 'We've always done it that way.' Critical thinking is one of the key components to overcoming the continued use of animals," advised Warren Casey, PhD, of the National Institute of Environmental Health Sciences (NIEHS) at the National Institutes of Health (NIH), during his keynote address at the virtual Summer School on Innovative Approaches in Science. The five-day event, held June 22-26, 2020, focused on educating a new generation of scientists on cutting-edge methods to reduce and replace animal use in toxicology and biomedical research.

The program was jointly hosted by the Physicians Committee for Responsible Medicine (PCRM), Johns Hopkins University Center for Alternatives to Animal Testing (CAAT), and the European Commission Joint Research Centre (JRC). The event featured over 45 speakers[1] from top institutions including Johns Hopkins University, Harvard University, the NIH, the US Environmental Protection Agency (EPA), PCRM, and more.

Over 600 international students and early-career researchers tuned in for a comprehensive program that included lectures, interactive sessions, e-poster presentations, laboratory and software demonstrations, and virtual attendee engagement.

The Summer School was originally planned as an in-person event to be held at the Johns Hopkins University School of Public Health in Baltimore, Maryland. However, due to the ongoing COVID-19 pandemic, the organizers transitioned to a virtual event. This opened the event to a wider audience and permitted people from around the world to participate without financial or travel constraints.

2 Critical thinking to advance science

Kristie Sullivan, PCRM's Vice President of Research Policy and **Thomas Hartung**, Director of CAAT, opened the first session of the Summer School addressing critical thinking skills as a fundamental tool in science. As **Warren Casey** highlighted in his keynote speech: "Science requires the critical use of reason to overcome biases and prejudice in experimentation and theory confirmation," the problem of institutionalized bias is one of the main reasons for continued use of animals, not only in toxicology but also in basic and applied research and in education and training. We have to understand the true limitations of animal experiments. This was addressed by **Kathrin Herrmann** from CAAT and **Francesca Pistollato** from JRC, who discussed the question: "Does animal experimentation still have a place in 21st century science?" Despite mounting evidence that animals are poor models for human disease, they explored how institutional and prevalent psychological tendencies – confirmation bias, cognitive dissonance, and motivated reasoning – impede scientific progress towards human biology-based models. The session was concluded by **Ursula Bosch** from the R3 Center for Innovation in Science Education, who focused on how philosophy in science education provides a framework for critical thinking that is crucial for rigorous research methodology. The panel discussion gathered thoughts from the speakers and also the attendees via real-time polling. After the discussion, most of the students supported a change in approach for biomedical research and toxicology (Fig. 1, 2).
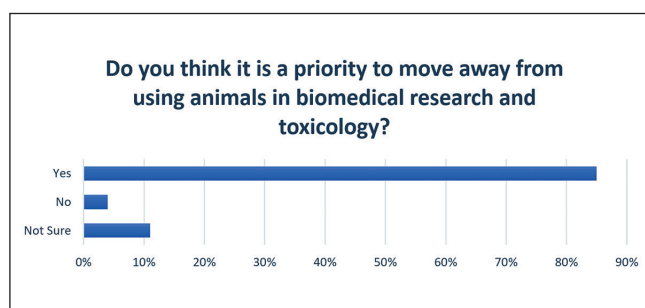

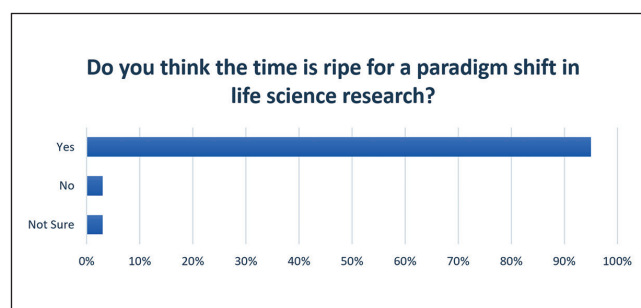**Fig. 1: Poll results collected in real time over the web via Slido[2]**

Fig. 2: Poll results collected in real time over the web via Slido[2]

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[1] Full list of speakers: https://www.ascctox.org/innovativescience2020

[2] https://www.sli.do/

Tab. 1: Biomedical science track session chairs, speakers, and lecture titles

In vitro and *in silico* approaches	Integrated approaches	Clinical relevance
Chair: Charu Chandrasekera, PhD, Canadian Centre for Alternatives to Animal Methods	Chair: Kambez Benam, DPhil, University of Colorado, Anschutz Medical Campus	Chair: Martin Stephens, PhD, CAAT, Johns Hopkins University
Virtual Patients: The Impending Revolution in Drug Development Himanshu Kaul, DPhil, University of British Columbia	**Disease-in-a-Dish: Moving Beyond Animal Models in Biomedical Research** Charu Chandrasekera, PhD, Canadian Centre for Alternatives to Animal Methods	**Birth Tissue as a Plentiful Resource for Primary Human Cells for Use in Research and Development** Sharon Presnell, PhD, Amnion Foundation
Xenofree Stem Cell Cultures to Study Diversity Factors of Disease Ann Lam, PhD, Physicians Committee for Responsible Medicine	**The Need to Prioritize "Replacement" in Alzheimer's Disease Research** Francesca Pistollato, PhD, European Commission Joint Research Centre	**Solving the Human Translation Problem for Cardiovascular Drug Discovery** Misti Ushio, PhD, TARA Biosystems
Modeling Human Lung Diseases in Three-Dimension: Introducing Small Airway-on-a-Chip and Breathing-Smoking Lung-on-a-Chip Microfluidic Technologies Kambez Benam, D Phil, University of Colorado, Anschutz Medical Campus	**Real-World Data Science to Untangle Environment and Genetics in Phenotype** Chirag Patel, PhD, Harvard Medical School	**Phase-0 Including Microdosing Approaches Using *In-Vivo* Human Data in the Preclinical Selection Process** Tal Burt, MD, Phase-0/Microdosing Network
3D Bioprinting as an *In Vitro* Platform for Complex Tissue Engineering Bhushan Mahadik, PhD, NIBIB/NIH Center for Engineering Complex Tissues, University of Maryland	**3D Organotypic Tissue Cultures as Predictive Human Disease Models** Helena Hogberg, PhD, CAAT, Johns Hopkins University	**Scientific Validity of Animal Free Antibodies for Use in Research and Risk Assessment** Rebecca Clewell, PhD, 21st Century Tox Consulting
Microphysiological Models: Challenges and Future Directions Šeila Selimović, PhD, U.S. Department of Health and Human Services	**Public/Private Development Model for Mini-Brain Technology** Lowry Curley, PhD, Axosim	**The Urgent Need for Human-Specific Research: Widespread Failures of Animal Models Mean They're Not an Option** Jarrod Bailey, PhD, Center for Contemporary Sciences

3 Lectures outlined latest innovations

To address the diverse needs of students, the Summer School offered two tracks – toxicology and biomedical science, featuring specific applications of current innovative nonanimal methods.

3.1 Biomedical science
The biomedical science track featured 15 speakers from academia, industry, and research organizations, who covered three main sessions; *in vitro* and *in silico* approaches, integrated approaches, and clinical relevance (Tab. 1). Throughout these sessions, speakers discussed the limitations of animal experiments to study human disease and the benefits of modern, human-relevant approaches.

Basic and applied research account for 68% of animals used, whereas regulatory use accounts for 23% (Zuang et al., 2019). Human-relevant, nonanimal approaches and technologies, such as patient-derived cells, large clinical data repositories, computational and imaging tools, machine learning, and microdosing approaches are enabling scientists to incorporate human relevance into biomedical research models and approaches. In the *in vitro* and *in silico* approaches session, speakers set the stage for the track by providing an overview of how some of these tech-

nologies are being used in a variety of applications for biomedical research. Attendees heard from scientists including **Kambez Benam**, who has created microfluidic technologies like the Small Airway-on-a-Chip and Breathing-Smoking Lung-on-a-Chip to model and study human lung diseases and **Bhushan Mahadik**, who uses 3D bioprinting and tissue engineering to develop novel, transplantable, and clinically relevant constructs, such as 3D placental models to study preeclampsia.

Speakers in the integrated approaches session focused on the importance of integrating multiple methods – including biological and computational methods – to elucidate molecular mechanisms underlying complex, multifactorial human diseases and address key needs and challenges in biomedical research. **Helena Hogberg** discussed her group's work on developing a 3D brain model from human induced pluripotent stem cells with different genetic backgrounds that has been used for numerous applications, including Parkinson's disease, cancer, autism, Down's syndrome, and Zika virus. The session also featured **Chirag Patel**, who discussed how biobank-derived cohorts and large-scale metagenomic data can be used to study disease and environmental factors simultaneously.

To improve the bench-to-bedside translational gap, human biology must serve as the gold standard. The clinical relevance ses-

Tab. 2: Toxicology track session chairs, speakers, and lecture titles

In vitro and *in silico* approaches	Integrated approaches	Regulatory application and risk assessment
Chair: Lena Smirnova, PhD, CAAT, Johns Hopkins University	Chair: Esther Haugabrooks, PhD, Physicians Committee for Responsible Medicine	Chairs: Maureen Gwinn, PhD, DABT, Environmental Protection Agency and Francesca Pistollato, PhD, European Commission Joint Research Centre
Nonanimal Testing Methods for Acute Exposures to Ocular, Dermal, Mucosal and Respiratory Epithelia Hans Raabe, MS, Institute for In Vitro Sciences	**Overview of Evidence Integration and International Collaboration in Toxicology** Nicole Kleinstreuer, PhD, NICEATM, National Institutes of Health	**Inhalation Risk Assessment Using Computational Fluid Dynamics and Three-Dimensional Respiratory Tissue** Doug C Wolf, DMV, PhD, Syngenta
Use of Microphysiological Systems for Toxicology Talita Marin, PhD, LNBio/CNPEM	**A Case Study: Using New Approach Methods to Assess Estrogen Receptor Activity**, Richard Judson, PhD Environmental Protection Agency	**Recent Advances in Animal Alternatives for Environmental Risk Assessment** Michelle Embry, PhD, Health and Environmental Sciences Institute
Developmental Neurotoxicity and 3D Human Cell Models Helena Hogberg, PhD, CAAT, Johns Hopkins University	**Understanding Extrapolation Models: Can We Relate Our Health to Tests in Cells?** Andy Nong, PhD, Health Canada	**Experimental Variability and Uncertainty in the Context of New Approach Methodologies for Potential Use in Chemical Safety Evaluation** Prachi Pradeep, PhD, US EPA ORISE Fellow
A Large Multitask Neural Network for Chemical Hazards Thomas Luechtefeld, PhD, Insilica	**Introduction to AOPs/MOA** Bette Meek, PhD, University of Ottawa	**Regulatory Perspectives on Nonanimal Approaches for Chemical Assessment in Health Canada** Francina Webster, PhD, Health Canada
Science Implementation: The Regulatory Context for New Approach Methodologies (NAMs) in Toxicology Kristie Sullivan, MPH, Physicians Committee for Responsible Medicine	**AOP Interactive Segment: Find the Key Events** Bette Meek, PhD, University of Ottawa Noffisat Oki, PhD, AAAS STPF Fellow, National Institutes of Health	**Risk Assessment and Alternative Approaches: Considerations in Latin America** Mauricio Rodriguez, PhD, CropLife Latin America

sion highlighted how human biology-based methods can bypass the perceived need for animals in research and how scientists are using innovative methods to answer their research questions. **Misti Ushio** presented a platform that produces engineered cardiac tissue with adult-like human physiology, which more accurately and quickly predicts the efficacy and safety of new medicines in early development. Participants also heard from **Tal Burt**, who discussed the benefits of microdosing to evaluate sub-therapeutic doses of new drugs in first-in-human studies. This approach increases safety, can improve the quality of preclinical candidates by providing human *in vivo* data to inform candidate selection, and can significantly reduce the number of animals used in drug development (Burt et al., 2020).

3.2 Toxicology
The toxicology track featured three main sessions: 1) *in vitro* and *in silico* approaches, 2) integrated approaches, and 3) regulatory application and risk assessment (Tab. 2).

The goal of the *in vitro* and *in silico* approaches session was to provide attendees with an introduction to available innovative nonanimal tools in the context of regulatory decision-making, including acute exposures and developmental neurotoxicity. **Talita Marin** used the Brazilian National Network of Alternative Methods (RENAMA) to demonstrate how nonanimal meth-

ods are implemented and showcased the development of various microphysiological systems fitted for regulatory applications. **Thomas Luechtefeld** demonstrated the UL Cheminformatics Tool Kit, which models 74 endpoints from chemical structural data, and discussed validation of computational approaches for chemical hazards.

The integrated approaches session aimed to address how to incorporate multiple data streams and, through the tools presented, highlight the importance of improving predictive safety assessments by integrating tools for critical areas like hazard assessment and internal dose extrapolation. **Nicole Kleinstreuer** overviewed how evidence integration approaches (e.g., weight of evidence (WoE), integrated approaches to testing and assessment (IATA), and defined approaches) are used in international collaborations, not only to strengthen safety assessments but also to generate robust toxicology predictions. To close the session, **Bette Meek** and **Noffisat Oki** led an interactive illustration of building an adverse outcome pathway (AOP) and identifying key events.

The objectives of the regulatory application and risk assessment session were to display how nonanimal models are developed and implemented within a regulatory context, to provide an understanding of decision-making requirements, and to demonstrate how nonanimal approaches can bridge the gap. The session started by highlighting the innovation and collaboration re-

quired to apply new methods to risk assessment. **Douglas C. Wolf** established the importance of problem formulation when developing appropriate alternative methods and then demonstrated the use of a reconstructed 3D human respiratory tissue combined with computational fluid-particle dynamics models for human health risk assessment of the pesticide chlorothalonil. Other examples of the implementation of new approach methodologies (NAMs) in different regulatory contexts were presented, including alternatives used for environmental risk assessments, high-throughput and other nonanimal data used for risk prioritization in Health Canada, and the development of emerging risk assessment schemes in Latin America. Using repeat dose toxicity studies and *in silico* models, **Prachi Pradeep** gave an overview of uncertainty and variability analysis, which is now more frequently integrated into the generation and evaluation of robust NAMs for regulatory application.

4 Interactive learning and career development

In addition to the scientific lectures, the numerous opportunities for student development and engagement were a key feature of the Summer School.

Voted among the favorite parts of the Summer School by participants were a total of six laboratory tours and software demonstrations. **Hans Raabe** from the Institute for In Vitro Sciences (IIVS) provided a virtual tour of its contract research laboratory focused upon *in vitro*, *in chemico*, and *ex vivo* test methods for evaluating product safety and chemical toxicology. **Brian Caffo**, professor of Biostatistics and Biomedical Engineering at Johns Hopkins University gave two tours. The Statistical Methodology and Applications for Research in Technology group has a particular focus on neuroimaging but also wearable and implantable technology and other biosignals. The goal of the group is to fuse high-end data analysis and statistical tools with processing and computational tools. The Data Science Lab in the Johns Hopkins Department of Biostatistics focuses on the development and application of data science, especially in public health, biology and medicine. **TJ Bozada** introduced the students to Sysrev, a collaborative, web-based platform for document review and data extraction. Sysrev's aim is to support any type of data extraction task. **Helena Hogberg** gave a virtual tour of CAAT's lab, in which she demonstrated the steps in generating the 3D brain model from monolayer neural precursor cells and showed some of the characterization CAAT has performed on the model. **Tony Williams** from EPA introduced the participants to the Computational Toxicology Program that utilizes computational and data-driven approaches integrating chemistry, exposure and biological data to help characterize potential risks from chemical exposure.

Two in-depth training sessions were available to attendees and the public during the week. **Rob Wright** from Johns Hopkins University Welch Medical Library conducted a comprehen-sive training on *The Mechanics of Expert Literature Searching*, where he introduced a PubMed search-building workflow that uses controlled vocabulary and other advanced searching capabilities to generate literature searches that aid in the reduction of animal use in science. The second training was hosted by **Maria Baltazar** and **Gavin Maxwell** from Unilever, who presented a case study on next generation risk assessment (NGRA) (Baltazar et al., 2020). This interactive session presented the process of a NGRA approach by describing the NAMs used and steps followed, i.e., existing information collation, exposure estimation, *in vitro* biological activity characterization, metabolism refinement, and margin of safety calculations. Participants were able to give feedback and ask questions via MentiMeter[3].

Effective science communication is fundamental for sharing recent findings and excitement for science, increasing public appreciation of science, increasing knowledge and understanding of science, and influencing the opinions, policy preferences or behavior of people (National Academies of Sciences, Engineering, and Medicine, 2017). This was made apparent during the presentation of **Denise-Marie Ordway**, Harvard Kennedy School Shorenstein Center on Media, Politics and Public Policy, on *Communicating Science to Journalists*. Ms. Ordway explained the news reporting process and offered guidance on how scientists can help journalists cover science and health topics clearly and accurately.

One main obstacle students and early-career researchers face when pursuing nonanimal research is the lack of research funding available. **Sue Leary**, Alternatives Research and Development Foundation, **Lucie Low**, National Center for Advancing Translational Science, and **Lingamanaidu Ravichandran**, NIEHS, participated in a panel discussion to address research funding for human-relevant methods. Through resources and examples of human-relevant research their organizations funded, panelists provided advice on how to find funding opportunities and structure grant applications. Panelists discussed challenges grant applicants face and the current research funding climate for early-career researchers using human-relevant methods and responded to questions from attendees.

Because the Summer School was entirely a virtual event, it was essential to include networking opportunities and stimulate attendee interaction. Virtual polls and quizzes were generated directly from the material presented throughout the sessions and integrated into the presentations and trainings. Attendees were encouraged to participate to win engagement prizes. We also held two virtual networking sessions, including an innovative science happy hour and a virtual mentoring session, where small groups of attendees met with presenters and other experts representing a wide range of scientific areas and career paths.

Applicants had the option to submit an abstract to be considered for a poster presentation session slot; the top forty were selected to give a two-minute flash poster presentation. Awards were given to poster presentations that scored the highest based on organization, overall quality, clarity of communication,

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[3] https://www.mentimeter.com/

and scientific impact. Poster presentations were also used as a criterion for two sponsored prizes to support early-career researchers in nonanimal innovative science. The Early Career Researchers Advancing 21st Century Science (ERA21) Recognition Award was presented to Lubnaa Hossenbaccus, Queen's University, for the poster *Evaluation of the Environmental Exposure Unit as an appropriate model to study house dust mite induced allergic rhinitis*. The PETA International Science Consortium Ltd. Award for Innovative Approaches in Science was awarded to Viviana Stephanie Costa Gagosian, Federal University of Paraná, for the poster *Development of a new reconstructed human epidermis equivalent and performance as skin irritation model*.

5 Maintaining momentum for innovative science

Numerous resources are available for students and researchers interested in human-focused university courses, scientific literature, hands-on training, networking groups, and more. These resources, and events like the Summer School, are essential to continue to educate the next generation of scientists on the need to shift away from animal use in the life sciences. As science continues to advance, hands-on training and direct communication will be essential to overcoming biases against more innovative nonanimal approaches.

PCRM provides two main training resources: the ERA21 program, which works with students and emerging scientists and connects them to the benefits of human-relevant research, and the NAMs Use for Regulatory Application (NURA) continuing education program, which provides NAMs training to industry, government, and academic scientists. CAAT also offers numerous resources to provide a better, safer, more humane future for people and animals. Opportunities from CAAT include the Next Generation Humane Science Award, the CAAT grants program, and free online academic courses in toxicology and humane science. The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) at the JRC is also committed to providing education and training resources that support the 3Rs approach: Replacement, Reduction and Refinement of animal procedures used for scientific purposes. Additionally, they host a biennial summer school in Ispra, Italy that focuses on nonanimal approaches in science, as well as offers traineeships for early-career researchers.

While there will be challenges ahead, strides are being made for innovative science. We are all accountable for maintaining the momentum, breaking down barriers, and providing the tools so that our next generation of scientists can pursue careers doing lifesaving work, without the use of animals.

Recordings from the Summer School on Innovative Approaches in Science and other materials are available online.[4]

References

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[4] https://pcrm.widencollective.com/portals/tgkpbij2/SummerSchool2020