CORESTA 2021 Smoke-Techno Virtu@l

SMOKE SCIENCE and PRODUCT TECHNOLOGY

Virtual Conference

SYMPOSIUM

Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction

19 October 2021

CORESTA

Version 210915

SYMPOSIUM

Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction

New approach methodologies (NAMs) represent *in vitro* and *in silico* or computational methodologies, an emerging set of chemical safety assessment tools, without needing additional *in vivo* animal testing.

This virtual Symposium introduces the current status of NAMs and their potential to support the evaluation of potentially reduced-risk tobacco products in support of tobacco harm reduction. Significant progress has been made toward the reduction, refinement, and replacement (3R) of animal studies for pharmaceutical and environmental toxicity assessment through the adoption of NAMs. At the same time, NAMs acceptance beyond screening and prioritization, including regulatory decision making, remains challenging.

At this Symposium, external experts from regulatory agencies and research organizations will share insights on the current status, strengths, and opportunities in application of NAMs using case examples from safety assessments of chemicals and consumer products. Following the presentations, the panel will be available for questions from the Symposium participants and discussion on opportunities and challenges in applying NAMs for toxicological assessment of tobacco and novel nicotine products.

The Symposium intends to foster scientific engagement between CORESTA members (endorsed by the CORESTA *In Vitro* Toxicity Testing Sub-Group, Biomarker Sub-Group, and 21st Century Toxicology for Next Generation Tobacco and Nicotine Products Task Force) and the external NAMs community, together facilitating the utility of NAMs in support of evidence-based tobacco regulatory science. Highlights of the Symposium will be submitted for a peer-review publication.

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The Symposium is being organised and led by Dr K.M. Lee from Altria Client Services, and Dr S. Bell from Integrated Laboratory Systems.

The Symposium will begin with a short welcome by the CORESTA Secretary General and an introductory talk by the organisers. It will continue with six 25-minute presentations that will include a speaker introduction and a five-minute Q&A. It will end with a 20-minute discussion with all the speakers.

TUESDAY 19 OCTOBER

SYMPOSIUM
Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction

Chair: K. Monica LEE
Co-Chair: Shannon BELL

PART 1

CET Time Zone		
13:30-13:35	**Welcome**	COLARD S. *CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France*
13:35-13:45	**NAM 00 Intro**	**Advancing new alternative methods for tobacco harm reduction** LEE K.M.(1); BELL S.(2) *(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* *(2) Integrated Laboratory Systems, 601 Keystone Park Drive, Suite 200, Morrisville, NC 27560, U.S.A.*
13:45-14:10	**NAM 01**	**US federal efforts to develop and implement alternatives to animal testing** KLEINSTREUER N. *NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), U.S.A.*
14:10-14:35	**NAM 02**	**Application of biokinetic modelling for *in vitro* to *in vivo* extrapolation (IVIVE) in chemical risk assessment** PAINI A.(1); WORTH A.(2) *(1) esqLABs GmbH, Hambierich 34, 26683 Saterland, Germany* *(2) European Commission Joint Research Centre (JRC), Ispra, Italy*
14:35-15:00	**NAM 03**	**Inhalation exposure modeling for assessing health risks of toxic aerosols and vapors** CORLEY R.A. *Greek Creek Toxicokinetics Consulting (GCTC), LLC, Boise, ID 83714, U.S.A.*

PART 2

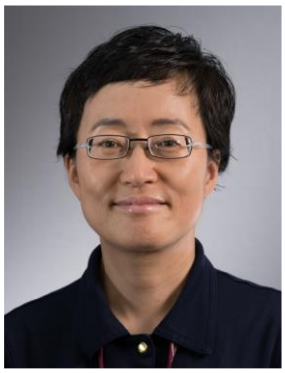
CET Time Zone		
15:10-15:35	**NAM 04**	**Assessing respiratory toxicity of chemicals in two human bronchial *in vitro* systems** STUCKI A.O. *PETA Science Consortium International e.V., Stuttgart, Germany*
15:35-16:00	**NAM 05**	***In silico* toxicology as a New Approach Methodology in tobacco regulatory science** VALERIO L.G. *FDA/Center for Tobacco Products, Office of Science, U.S.A.*
16:00-16:25	**NAM 06**	**Application of mechanistic data in risk assessment: exposure alignment and evidence integration** JARABEK A.M. *U.S. Environmental Protection Agency's Office of Research and Development (ORD), U.S.A.*
16:25-16:45	**Discussion**	LEE K.M.(1); BELL S.(2) *(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* *(2) Integrated Laboratory Systems, 601 Keystone Park Drive, Suite 200, Morrisville, NC 27560, U.S.A.*

NAM 00

INTRODUCTION: Advancing new alternative methods for tobacco harm reduction

LEE K.M.(1); BELL S.(2)

(1) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.
(2) Integrated Laboratory Systems, 601 Keystone Park Drive, Suite 200, Morrisville, NC 27560, U.S.A.



Dr. K. Monica Lee, Ph.D., D.A.B.T., is an Associate Fellow in Regulatory Sciences at the Altria Client Services LLC. Dr. Lee leads the Biological Science Insights group, identifying, building and utilizing innovative and pragmatic in vitro and in vivo toxicological evaluation approaches in support of product development, regulatory compliance and scientific engagement. She has extensive experiences in regulatory and foundational studies and published numerous peer-reviewed articles on the topics of reduced-risk disease models, comparative and mechanistic toxicology, in vitro-to-in vivo extrapolation and kinetic modeling. Prior to joining ALCS in 2015, she served as the BioScience Director at the JT International, Sr. Toxicologist at Battelle Toxicology Northwest, and Postdoctoral fellow at Pacific Northwest National Laboratories. Her PhD is in Pharmacology/Toxicology from the University of Georgia. Dr. Lee builds and promotes the use of in silico and in vitro-based predictive toxicological tools in support of tobacco regulatory science, proactively engaging with CORESTA members and external partners across research community. She is organizing the first NAM symposium at the SSPT 2021 Conference as an introduction to the CORESTA members and a starting point for future engagements and collaborations.

Dr. Shannon Bell leads the Computational Toxicity and Data Science group at Integrated Laboratory Systems, where she works with clients to support data-driven decision for product development and assessment. Her area of interest is in how to combine distinct data streams together to unlock new knowledge. In that vein, her group has aided clients in automated data retrieval, development of web-based data tools, bioinformatics including pathway- and adverse outcome-based data analysis, and development of in vitro to in vivo extrapolation approaches. Dr. Bell received her Ph.D. (dual) in Biochemistry and Molecular Biology with Quantitative Biology (Systems Biology focus) at Michigan State University in 2012 where her research focused on approaches for detection and prioritization of phenotypic variants from a high throughput phenotypic screen of Arabidopsis genetic variants. During her postdoc with the US EPA, Dr Bell continued her work supporting data integration and prioritization, pioneering the computationally predicted adverse outcome pathway (cpAOP) approach aimed at leveraging multiple information streams to rapidly generate putative AOPs to help inform chemical testing strategies. Dr Bell was the technical lead for the Integrated Chemical Environment (ICE) as part of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) contract from its inception until 2021. ICE is a web resource that houses and provides public access to data and tools aimed at facilitating the use of new approach methodologies (NAMs) to aid in chemical risk assessment.

NAM 01

US federal efforts to develop and implement alternatives to animal testing

KLEINSTREUER N.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), U.S.A.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is made up of representatives from US federal agencies that require or consider chemical safety testing data, and are interested in more rapid, human-relevant approaches to supplement or replace existing regulatory standard *in vivo* guideline tests. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) provides scientific and administrative support to ICCVAM through a variety of efforts including methods development and validation, construction of computational tools, communication and outreach, and stakeholder engagement, all driven by federal agency priorities and decision contexts. This talk will provide an overview of ICCVAM and NICEATM's progress in developing, evaluating, and implementing alternatives to animal testing. Emphasis will be on implementation of the ICCVAM strategic roadmap, ongoing efforts to replace the "six-pack" of acute toxicity tests, and development of computational resources such as *in vitro* to *in vivo* extrapolation workflows, with specific examples in the areas of inhalation toxicity testing.

Dr. Nicole Kleinstreuer is the acting director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the US federal resource for alternatives to animal testing, within the Division of the National Toxicology Program at the NIEHS. At NICEATM, she leads domestic and international efforts to develop novel testing and analysis strategies that provide more rapid, mechanistic, and human-relevant predictions of potential environmental chemical hazards. Kleinstreuer's research focuses on mathematical and computational modeling of biological systems and their susceptibility to perturbations that result in adverse health outcomes, and she has authored over 100 peer-reviewed publications in these areas. She holds a secondary appointment in the NIEHS Division of Intramural Research Biostatistics and Computational Biology Branch, and adjunct faculty positions in the Yale University School of Public Health and the Eshelman School of Pharmacy at UNC Chapel Hill.

NAM 02

Application of biokinetic modelling for *in vitro* to *in vivo* extrapolation (IVIVE) in chemical risk assessment

PAINI A.(1); WORTH A.(2)

(1) esqLABs GmbH, Hambierich 34, 26683 Saterland, Germany
(2) European Commission Joint Research Centre (JRC), Ispra, Italy

In vitro chemical safety testing methods offer the potential for efficient and economical tools to provide relevant assessments of human health risk. To realize this potential, methods are needed to relate *in vitro* effects to *in vivo* responses, without relying on *in vivo* animal testing. To this end, *in vitro* to *in vivo* extrapolation (IVIVE) is key. IVIVE is essentially representing two main streams: 1) Upscale of *in vitro* measurements from molecular reaction to full organ to be used in biokinetic-dynamic modelling (biological scaling), and 2) The linking of *in vitro* concentration response (endpoints/effect) to external exposure doses by means of biokinetic modelling. In the current presentation we will highlight the main concepts of IVIVE, report the main biokinetic models and illustrate the IVIVE approach using case studies that were published as part of the recent OECD PBK model Guidance document (OECD, 2021).

Dr Alicia Paini holds a degree in Food Science and Technology from the University of Parma, Italy. An MSc in Food Safety and a PhD in Toxicology from Wageningen University, The Netherlands, where she gained know-how in generation of data to develop physiologically based kinetic (PBK) models for genotoxic chemicals; she did her PhD research at the Nestlé Research Centre, Switzerland. She worked for nine years at the European Commission's Joint Research Centre (JRC) - EURL ECVAM on implementing and promoting in silico tools (biokinetic and dynamic modelling, e-health, etc) for policy and application in regulatory decision making. In October 2021 she joined esqLABS GmbH (Germany) as a Senior Scientist, leading the biokinetic working group of the EU-funded ONTOX project. She is a European Registered Toxicologist since 2013.

NAM 03

Inhalation exposure modeling for assessing health risks of toxic aerosols and vapors

CORLEY R.A.

Greek Creek Toxicokinetics Consulting (GCTC), LLC, Boise, ID 83714, U.S.A.

Computational fluid dynamics (CFD)-based approaches, either alone or coupled with lower dimensional models such as physiologically-based pharmacokinetic (PBPK) and multiple-path particle dosimetry (MPPD) models (multiscale models) have been developed to facilitate the reduction in animal testing in human health risk assessments for inhaled materials. Case studies will be presented where these *in silico* approaches were used to establish human equivalent exposure concentrations for cytotoxic pesticide aerosols corresponding to exposure conditions used *in vitro* in toxicity studies with human air way cells grown at air-liquid interface as well as for reactive vapor constituents of tobacco smoke previously studied in animal models. CFD-based modeling of respiratory dosimetry under realistic human exposure vs. experimental conditions improves the relevance of new risk assessment approaches that utilize human cells *in vitro* as well as historically relevant *in vivo* studies thereby reducing the overall use of animals in tobacco harm reduction research.

Dr. Richard A. (Rick) Corley is the sole proprietor of Greek Creek Toxicokinetics Consulting (GCTC), LLC and an Emeritus Laboratory Fellow (retired) from the Pacific Northwest National Laboratory (PNNL) where he established a multi-disciplinary research program in toxicokinetic modeling and multi-scale computational toxicology. Prior to joining PNNL in 1996, he rose from a Post-Doctoral Fellow to Group Leader of the Inhalation and Chronic Toxicology Groups and Toxicology Consultant for the Dow Chemical Company. His current research continues to focus upon the development and application of multi-scale toxicokinetic models used to characterize target tissue dosimetry following exposure to chemical, biological, or therapeutic agents across species and life stage. He has published over 100 peer-reviewed articles and over 85 book chapters, government reports and technical R&D reports in the areas of physiologically based pharmacokinetic (PBPK) modeling, computational fluid dynamics (CFD) based modeling of the respiratory system, toxicology, and human health risk assessments. Dr. Corley has served as President of the Biological Modeling Specialty Section of the Society of Toxicology, Program Committee of the Society of Toxicology, and multiple committees and advisory panels for the National Academies of Sciences, Environmental Protection Agency, Food and Drug Administration, Office of Technology Assessment, and the International Life Sciences Institute. Dr. Corley received his Ph.D. in Environmental Toxicology/Veterinary Biosciences in 1985 from the University of Illinois at Urbana-Champaign.

NAM 04

Assessing respiratory toxicity of chemicals in two human bronchial *in vitro* systems

STUCKI A.O.

PETA Science Consortium International e.V., Stuttgart, Germany

Risk assessment and management relies on approaches that can accurately and efficiently predict the toxicity of chemicals in humans. Inhalation is a major route by which exposure to substances can occur, and is an area where resources have been dedicated to optimize human-relevant *in vitro* approaches. In this study a two-dimensional (2D) human bronchial epithelial cell line (BEAS-2B) and a three-dimensional (3D) human reconstructed tissue model (MucilAir™, Epithelix) were used to predict the ability of chemicals to cause portal-of-entry effects on the human respiratory tract. The human cell-based systems were exposed to different concentrations of silanes (triethoxysilane (TES) and trimethoxysilane (TMS)) surfactants (Triton X-100 and oleoyl sarcosine) at the air-liquid interface in a VITROCELL® 6/4 exposure module. Nitrogen dioxide (NO_2) was included as a positive control and sodium chloride and clean air (CA) or nitrogen gas (N) as negative controls. Endpoints assessed include cell viability (Prestoblue™ assay), cytotoxicity (lactate dehydrogenase assay; LDH), and expression of inflammatory markers (electrochemiluminescence immunoassay, Meso Scale Discovery) and, in addition for the 3D tissues, morphology (hematoxylin and eosin (H&E) staining), barrier integrity (transepithelial electrical resistance, TEER), and cilia beat frequency (SAVA system). Preliminary studies demonstrated a concentration-dependent decrease in cell viability and an increase in cytotoxicity after 1 hour exposure of BEAS-2B cells to TES (0.72 ppm, 25 ppm, and 85 ppm) as compared to CA. A significant increase in expression of inflammatory markers including interleukin IL-6, IL-8, IL-2, and tumour necrosis factor-alpha (TNF-α), was observed at 25 ppm of TES. Studies are underway to assess the additional test chemicals and endpoints in both systems. The results of this project can be used to better understand the usefulness of different test systems and, therefore, help guide selection. They may also be used to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and inform regulatory decision-making.

Dr Andreas Stucki is an adviser to PETA Science Consortium International e.V. He received his doctorate in biomedical sciences from the University of Bern, Switzerland. His doctoral research focused on the development and biological evaluation of a breathing lung-on-a-chip – an advanced in vitro model of the human air-blood barrier. After his PhD, he worked on in vitro respiratory toxicology testing before returning to the University of Bern/AlveoliX for his postdoctoral research. With several years of experience in organ-on-a-chip and pulmonary research, he advises the Science Consortium on inhalation toxicity and nanomaterial testing issues.

NAM 05

In silico toxicology as a New Approach Methodology in tobacco regulatory science

VALERIO L.G.

FDA/Center for Tobacco Products, Office of Science, U.S.A.

In silico (computational) toxicology is a new approach methodology (NAM) that is an integral part of the US federal collaboration Toxicology in the 21st Century, and FDA's Predictive Toxicology Roadmap. *In silico* methods are efficient, reliable, cost effective, and align with the '3Rs' principals of animal testing. Smoke from combusted tobacco products contains thousands of organics including harmful or potentially harmful chemicals. The aerosol from electronic nicotine delivery systems (ENDS) is also a complex mixture of constituents. In addition, flavored e-liquids used with ENDS have many chemical constituents. Collecting hazard identification information for every tobacco constituent using conventional *in vivo* toxicology studies would be impractical. *In silico* methods are versatile showing capabilities in toxicity prediction screening, data mining, structural alert identification, model building and many other techniques. This presentation will discuss how different *in silico* approaches, if properly used and in context, have the potential to overcome the challenges with traditional testing models to generate useful data in the frame of tobacco regulatory science. Limitations and other considerations inherent to use of *in silico* approaches will also be discussed. FDA/CTP's research on utility of NAMs to support toxicology evaluations is illustrated by *in silico* screening for genetic toxicity hazard identification of tobacco constituents using (quantitative) structure-activity relationship ((Q)SAR) computer models, results from predictive computational model validation testing to classify mutagenic potential, and research on integration of *in silico* and *in vitro* high throughput (HTP) screening technologies to identify the genotoxic mode of action of flavor compounds. With focus on flavor compounds relevant to tobacco products, the *in vitro* research using clastogen-sensitive (γH2AX and p53) and aneugen-sensitive (p-H3 and polyploidy) biomarkers of DNA damage and machine learning algorithms can classify the genotoxic mode of action (structural chromosome damage, or aneuploidy). Such work underscores how leveraging different NAMs generates streams of evidence that can be productive to increase knowledge about toxicological hazards of compounds. As predictive models such as (Q)SARs and HTP multiplexed *in vitro* and machine learning methods are trained and garnered with empirical data, the quality of data used to make predictions as well as interpretations becomes paramount. Also, the integration of *in silico* and *in vitro* NAMs offers another approach to better understand toxicity profiles of constituents with the goal to increase the strength of evidence to make better decisions about potential toxicities that may lead to health risks.

Dr. Valerio is Associate Director of the Division of Nonclinical Science at FDA/Center for Tobacco Products, Office of Science. He has been with FDA for 17 years in review of regulated products and applied research and has leadership experience in the private sector in safety science of food ingredients, as well as aerosolized and topically applied products. He serves on FDA's Alternative Methods Working Group which aims to advance and catalyze the development and potential application of new approach methodologies to support regulatory toxicology at the agency. Current applied research he has fostered at FDA is in the utility of computational predictive toxicology models of molecular structure and cheminformatic approaches integrated with in vitro screening to speed hazard identification of compounds present in or emitted by tobacco products. His academic background is in biochemical metabolism and molecular toxicology with the US National Science Foundation and as a National Research Council Gastroenterology Fellow at the University of Colorado School of Medicine. His PhD is in Pharmaceutical Science from the University of Colorado.

NAM 06

Application of mechanistic data in risk assessment: exposure alignment and evidence integration

JARABEK A.M.

U.S. Environmental Protection Agency's Office of Research and Development (ORD), U.S.A.

Risk assessment relies on integration of evidence across several data streams by necessity. Conceptual constructs for source-to-outcome modeling based on aggregate exposure pathway (AEP) and adverse outcome pathway (AOP) frameworks can provide a mechanistic scaffold for evidence integration to now include application of new approach methods (NAMs). Based on the conceptual construct, this presentation will discuss the critical role of dosimetry models to provide exposure alignment across experimental platforms and illustrate impact by two recent integrated approaches to testing and assessment (IATA) under the Toxic Substances Control Act (TSCA) that formalized a role for NAMs. The IATA demonstrate how consideration of physicochemical properties and NAMs aimed at key events (KEs) of AOPs create context for evaluation of the need and strategy for higher-tiered testing based on mechanistic responses, dosimetry, and exposure information.

Dr. Annie M. Jarabek currently serves as a Senior Science Advisor in the Center for Public Health and Environmental Assessment (CPHEA) in the Research Triangle Park, within the U.S. Environmental Protection Agency's Office of Research and Development (ORD); following service as the Deputy Director of the Human Health Risk Assessment (HHRA) national research program in ORD. Throughout her career she has worked on high priority risk assessments, dosimetry models or analysis methods across all media and routes of exposure; always striving to bring mechanistic data into decision workflows. She has received a Lifetime Achievement Award from the University of Massachusetts, the Risk Practitioner of the year award from the Society for Risk analysis and the Lehman award for risk assessment from the Society of Toxicology as well as several best paper awards and several medals from the Agency.

Appendix: TIME ZONES

Time zone equivalents to CET 13:30 Conference start time

Timezones	City	Time	Hour difference with Paris
CET	**Paris**	**13:30**	**0**
PDT	San Francisco	04:30	**-9**
CST	Managua	05:30	**-8**
EDT	New York	07:30	**-6**
BRT	São Paulo	08:30	**-5**
BST	London	12:30	**-1**
CAT	Harare	13:30	**0**
EET	Bucharest	14:30	**+1**
GST	Dubai	15:30	**+2**
IST	New Delhi	17:00	**+3:30**
CST	Beijing	19:30	**+6**
JST	Tokyo	20:30	**+7**
AEST	Sydney	21:30	**+8**

CET Central European Time
PDT Pacific Daylight Time
CST Central Standard Time
EDT Eastern Daylight Time
BRT Brasilia Time
BST British Summer Time
CAT Central Africa Time
EET Eastern European Time
GST Gulf Standard Time
IST Indian Standard Time
CST China Standard Time
JST Japan Standard Time
AEST Australian Eastern Standard Time