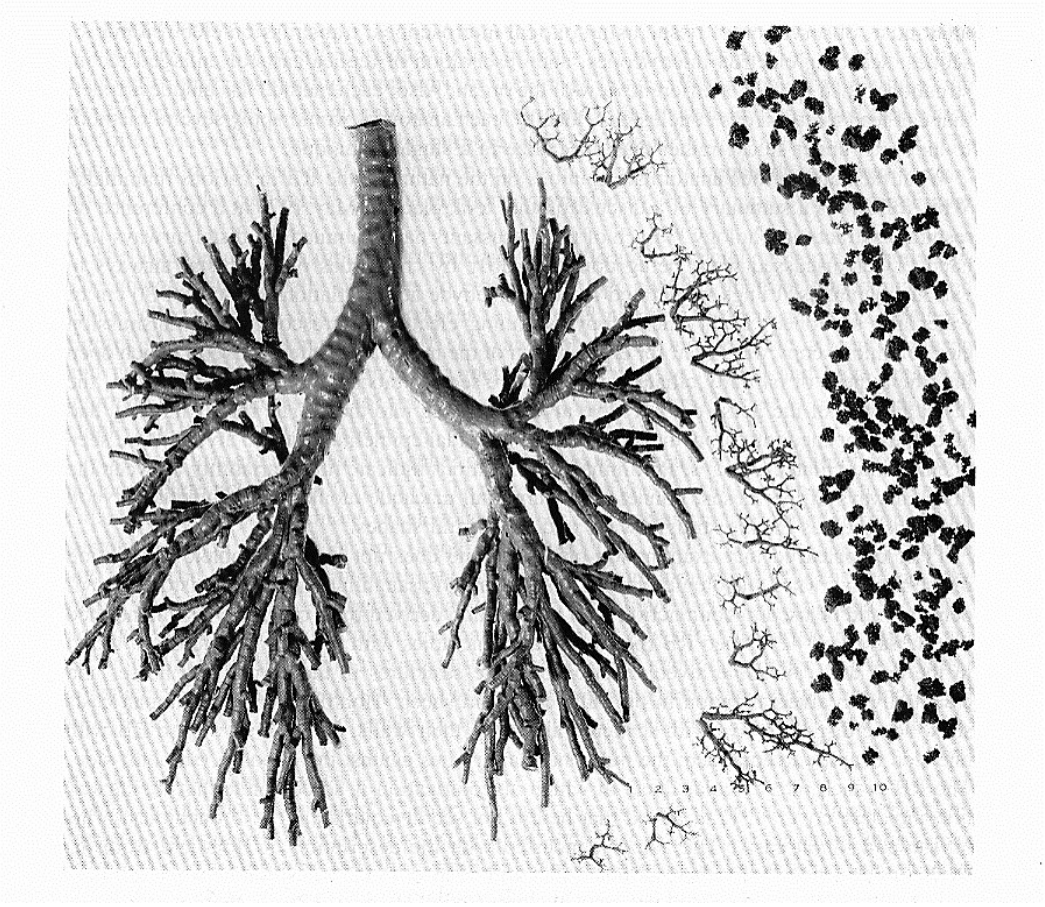
INHALED AEROSOL DOSIMETRY: MODELS, IMPLICATIONS AND IMPACT

PROCEEDINGS OF THE CONFERENCE

The Arnold and Mabel Beckman Center of the
National Academies of Sciences and Engineering

Irvine, California
October 16 - 18, 2024



Editors, Robert F. Phalen and Leslie Owens
Irvine, California January 2025
(Air Pollution Health Effects Laboratory Report, # 25-01)

October 16-18, 2024 conference attendees gather during their lunch break.

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AB-44 **Shamudra Dey** ***(Marquette University & Medical College of Wisconsin),*** Dry powder inhaler deposition in the larynx and the risk of steroid inhaler laryngitis: a computational fluid dynamics study

AB-45 **Chang Gyu Woo** ***(KOREATECH),*** Standardization of lung Microphysiological System

I. INTRODUCTION

1. The First Aerosol Dosimetry Conference

Aerosol dosimetry is a broad and important topic. For inhaled particles, the traditional concept of dose – which is the mass of an administered substance, sometimes normalized to body weight or surface area – is too simplistic. The particles in an inhaled aerosol can deposit on airway surfaces in complex patterns. Once deposited, the fates of particles depend on their physiochemical properties, their initial deposition sites, and the anatomy and physiology of the subject. Particles and their components may be cleared by mucociliary action, enter tissue fluids (e.g., blood and lymph) to be distributed throughout the body, or be taken up by cells and tissues in the respiratory tract. Aerosol dosimetry covers all of these dynamic events in order to understand the basic physiologic processes involved, and to predict health effects – both positive and negative. Advances in aerosol dosimetry are made by individuals from several disciplines, including chemists, physicists, atmospheric scientists, engineers, mathematicians, physiologists, toxicologists, pharmacologists, anatomists, risk-assessors, physicians, and veterinarians. These diverse specialists seldom meet together because they attend their own disciplinary meetings. The first conference was held in order to bring these specialists together to exchange ideas and to facilitate future research efforts. The "Frontiers in Aerosol Dosimetry Research" conference was held in 2005 at The Beckman Center of the National Academies in Irvine, California. There were 91 attendees from 12 countries, and 56 papers were presented. Peer reviewed papers were published in *Inhalation Toxicology* (18(10) 2006).

2. The Second Conference

The second Conference, "Advances in Aerosol Dosimetry Research, was also held at the Beckman Center of the National Academies in Irvine, California on October 24 and 25, 2014. It was attended by 58 scientists, including postgraduate and graduate students, from 5 countries. The attendees' affiliations included universities, research institutes, industry laboratories, government laboratories, and consulting firms. Fifty-six papers were presented, ranging from basic to applied topics. The papers were organized into eight sessions, including: Inhaled Aerosols; Posters (covering a large variety of topics); Tobacco Smoke Dosimetry; Computational Fluid Dynamics Modeling; Animal Models; Human applications; In-vitro Dosimetry; and Miscellaneous Topics. Abstracts of the papers are included in the Proceedings, along with commentaries, and submitted papers. Peer reviewed papers were published in the *Journal of Aerosol Science* (99, September 2016).

3. The Third Conference

The third conference, "Inhaled Aerosol Dosimetry: Models, Applications and Impact", was also held at the renamed, "The Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering", Irvine, California, October 10-12, 2019. It attracted 87 international attendees, and 65 papers and posters were presented. There were two inaugural sessions covering vitro aerosol exposures and systems. Keynote/Overview papers were delivered by Dr. Gunter Oberdorster, Dr. Roger McClellan, Dr. Mark Hoover, and Dr Annie Jarabek, Discussions and written comments produced several recommendations (see Phalen R.F., Hoover, M.D., Oldham, M.J., and Jarabek, A.M., Inhaled Aerosol Dosimetry: Research-related needs and recommendations. *Journal of Aerosol Science*, 155. Article Number 105755, June 2021). The peer-reviewed papers from the conference are published in a Virtual Special Issue, "Inhaled Aerosol Dosimetry of the *Journal of Aerosol Science*, 2021 (Phalen, R.F. and Owens-Kimura, L., Eds., *Inhaled Aerosol Dosimetry: Proceedings of the Conference*, UCI Dept. of Medicine, UCI Department of Environmental and Occupational Health (School of Public Health) 2021.

4. The Fourth Conference

The fourth conference, "Inhaled Aerosol Dosimetry: Advances, Applications and Impacts on Risk Assessments and Therapeutics", was also held at "The Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering", Irvine, California, October 16-18, 2024. It attracted 66 attendees including graduate students from seven nations (USA, Germany, France, India,

the Netherlands, Switzerland, and S. Korea) and 52 papers and posters were presented. There were two highlighted sessions, number 5 and 6 in the program, on in vitro aerosol exposures and exposure systems. Keynote/Overview papers were delivered by Dr. Annie Jarabek (U.S. EPA), Dr. Gunter Oberdorster (U of Rochester), Dr. Jesssica Murray (U.S. EPA), Dr. Roger McClellan (Toxicology and Risk Analysis), Dr. Ross Walenga (U.S. FDA), and Dr. Ron Wolff (RK Wolff Safety Consulting, Inc.). See abstracts AB 52, 03, 04, 34 & 39, 50, and 41 respectively for these presentations. There were three awards to students for Outstanding Research, and a hosted luncheon where graduate students had open discussions with established scientists representing academia, government, pharmaceutical industry, and consultants. Discussions and written comments after the last session produced several recommendations that are to be published. And peer-reviewed papers from the conference are to be published in a Virtual Special Issue, "Inhaled Aerosol Dosimetry of the *Journal of Aerosol Science,* edited by Dr. Phalen (UC Irvine), Dr. Darquenne (UC San Diego), and Dr. Golshahi (Virginia Commonwealth University). The concluding open discussion addressed the need for another future conference and for the attendees to increase their collaborations.

5. Sponsors

NIH/NIEHS, U.S. EPA, ISAM, UC Irvine Advancement, and The Air Pollution Health Effects Laboratory, The Department of Environmental Health in The Joe C. Wen School of Population & Public Health, and the Department of Medicine, Division of Occupational and Environmental Health.

6. Organizers, Session Chairs and Co-Chairs, and Student Volunteers

Program and Organizing Committee Members, and Session Chairs/Co-Chairs (*)

Flemming Cassee (RIVM, Netherlands)
Rick Corley (Greek Creek Toxicokinetics Consulting)
Chantal Darquenne (UC San Diego)
Andrea De Vizcaya-Ruiz (UC Irvine)
Laleh Golshahi (Virginia Commonwealth University)
Mark Hoover (Mark D Hoover LLC)
Annie Jarabek (US EPA)
Chong Kim (Retired)
Michael Kleinman (UC Irvine)
Loyda Mendez (Universidad del Este, Puerto Rico)
Jessica Oakes (Northeastern University)
Michael Oldham (Oldham Associates LLC)
Robert Phalen (UC Irvine)
Jonathan Phillips, (Amgen)
Otmar Schmid (Helmholtz Zentrum Munchen)
Jin Xiang Xi (University of Massachusetts Lowell)
Günter Oberdörster, (URMC Rochester)*
Ron Wolff, (RK Wolff Safety Consulting Inc.)*

Student Volunteers

Alejandro Martinez Santos (UC Irvine)
Arturo Jimenez Chavez (UC Irvine)
Desiree Mills (UC Irvine)
Elizabeth Choy (UC Irvine)
Jayveeritz Bautista (UC Irvine)
Russell Morales Rubio (UC Irvine)
Charbel Yazbeck (Northeastern University)
Mohammad Hejazi (Virginia Commonwealth University)
Taylor Jefferis (Baylor University)
Yanira Baldovinos (Baylor University)

7. Publications

Two publications are being generated by the Conference; this Proceedings and the *Journal of Aerosol Science* Virtual Special Issue on “Inhaled Aerosol Dosimetry “(2025).

Inhaled Aerosol Dosimetry
October 16-18, 2024
Irvine, California

♦ PROGRAM ♦

Wednesday, October 16
Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering

8:00 am – 9:00 am Hosted Breakfast (Registration all day) and Poster Set-up

9:00 am – 9:15 am Welcome and Introduction

9:15 am – 10:30 am **Session 1:** ***Overviews***

Co-Chairs: **Mark Hoover*, (Mark D Hoover LLC)***
Flemming Casee, *(RIVM, Netherlands)*

1.1 **Annie M. Jarabek *(US EPA)*,** Modernizing Workflows for Environmental Inhalation Risk Assessment: Challenges and Research Needs to Support Coherent Evidence Integration - (AB-52)
1.2 **Günter Oberdörster *(URMC Rochester),*** Unfinished Business in Particle Inhalation Dosimetry - (AB-03)
1.3 **Jessica Murray *(US EPA),*** Challenges and Progress in Characterizing In Vitro Dosimetry for Air-Liquid Interface Exposures - (AB-04)
1.4 **Roger O. McClellan *(Toxicology and Risk Analysis)*,** Inhaled Aerosol Dosimetry in the Context of a Source-Exposure-Dose-Health Effects Paradigm - (AB-34)

10:30 am – 10:55 am Refreshment Break

10:55 am – 12:10 pm **Session 2:** ***Applications***

Co-Chairs: **Otmar Schmid, *(Helmholtz Munich)***
Jinxing Xi, *(University of Massachusetts)*

2.1 **Ross Walenga *(U.S. Food and Drug Administration)*,** In Silico Modeling to Support Development and Approval of Generic Orally Inhaled Drug Products in the United States - (AB-50)
2.2 **Ron Wolff *(RK Wolff Safety Consulting Inc.)*,** Influence of Aerosol Deposition, Molecular Weight and Nonclinical Studies on Inhaled Biologics Development - (AB-41)
2.3 **Ignacio R. Bartol *(Georgia Institute of Technology)*, M.S. Graffigna, M. Tano, S.A. Dewji,** Computational Framework for Subject-specific Aerosol Dosimetry and Internal Radiation Dosimetry using CFPD and Monte-Carlo Transport Codes - (AB-46)
2.4 **Roger O. McClellan *(Toxicology and Risk Analysis)*,** Aerosol Science Development Fostered by the Manhattan Project, Atomic Energy Commission and Department of Energy Programs - (AB-39)

12:10 pm – 1:10 pm Hosted Lunch and Group Photograph

1:10 pm – 3:05 pm **Session 3:** ***Modeling***

Co-Chairs: **Chantal Darquenne, *(University of California Irvine, San Diego)***
Laleh Golshahi, *(Virginia Commonwealth University)*

3.1 **Bahman Asgharian *(Applied Research Associates, Inc.)*, O. Price, A.T. Borojeni, A.P. Kuprat, R. Singh, R.A. Corley, C. Darquenne.** Modeling Aerosol Bolus Dispersion and Deposition in the Human Lung **-** (AB-01)
3.2 **Robert F. Phalen *(UC Irvine)*, M.D. Hoover and R.O. McClellan**, Updating Aerosol Inhalability - (AB-02)
3.3 **Guilherme J.M. Garcia (*Marquette University and The Medical College of Wisconsin)*, J. Wu, W.D. Bennett, R.L. Walenga, J.S. Kimbell, J.D.**

Schroeter, Validation of Computational Fluid Dynamics Simulations of Nasal Sprays with Regional Doses Measured by Gamma Scintigraphy - (AB-11)

3.4 **Debjit Kundu *(Indian Institute of Technology Madras)*, M.V. Panchagnula,** Monte-Carlo Simulations of Stochastic Asymmetric Bronchial Trees: Towards Targeted Pulmonary Drug Delivery - (AB-15)

3.5 **Francesco Lucci (*PMI R&D*), A.K. Kuczaj,** AeroSolvedSystem: Novel Approach for Aerosol Dosimetry Calculations - (AB-28)

3.6 **Lin Yang *(Helmholtz Munich)*, M. Blayac, O. Schmid**, AI-Driven Aerosol Dosimetry and Regional Distribution in Murine Lungs - (AB-38)

03:05 pm – 3:30 pm Refreshment Break

3:30 pm – 5:25 pm **Session 4: *Children & Upper Airways***

Co-Chairs: Jessica Oakes, *(Northeastern University)*
Loyda Mendez, *(Universidad del Este, Puerto Rico)*

4.1 **Laleh Golshahi *(Virginia Commonwealth University)*, M. Hejazi, X. Owen, J. Wilkins, T. Schuman, M. Hindle, W. Longest, R. Walenga, A. Kaviratna, B. Newman,** Anatomically-Realistic Adult and Pediatric Nasal Models as Platform Tools to Study Regional Drug Delivery and Bioequivalence of Suspension Nasal Sprays - (AB-14)

4.2 **Jana Kesavan, *(U.S. Army Combat Capabilities Development Command Chemical Biological Center)*, B.L. Laube, G. Farias, N. Karavas, M. Blondel, J. Suman,** Targeting Aerosol Delivery to Regions of Nasal-Associated Lymphoid Tissue in Three Dimensional Models of Human Intranasal Airways Using the BiVax Intranasal Atomizer - (AB-10), and **7.2**

4.3 **Uschi M. Graham, *(URMC Rochester)*, G. Oberdörster,** The Need for Dosimetry to Model Particle-Cell Interactions Following Their Nose-to-Brain Translocation - (AB-22)

4.4 **Shamudra Dey, *(Marquette University & Medical College of Wisconsin)*, J.M. Bock, G.J.M. Garcia,** Dry Powder Inhaler Deposition in the Larynx and the Risk of Steroid Inhaler Laryngitis: A Computational Fluid Dynamics Study - (AB-44), 7.18

4.5 **Flemming Cassee *(RIVM, Netherlands)*, E. Bongaerts, A. Couturier-Tarrade, L. Campagnola,** Placental-Fetal Distribution of Inhaled Carbon and Silver Nanoparticles in a Pregnant Rabbits and Mice, Respectively - (AB-23)

5:30 pm Busses to Hotels

Thursday, October 17
Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering

8:00 am – 9:00 am Hosted Breakfast

9:00 am – 10:20 am **Session 5: *In Vitro***

Co-Chairs: Ron Wolff, *(RK Wolff Safety Consulting Inc.)*
Jonathan Phillips, *(Amgen)*

5.1 **Detlef Ritter (*Fraunhofer Institute for Toxicology and Experimental Medicine ITEM)*, K. Schwarz, W. Koch,** Dosimetry Concepts for In Vitro Prediction of Respiratory Toxicity. **Part 1**: Identification of Relevant Dose-Metrics, Case Study Using a 2-Stroke Engine Exhaust - (AB-05)

5.2 **Katharina Schwarz *(Fraunhofer Institute for Toxicology and Experimental Medicine ITEM)*, D. Winterberg, H. Obernolte, S. Wronski, T. Hansen, D. Ritter, K. Sewald, A. Braun,** Dosimetry Concepts for In Vitro Prediction of Respiratory Toxicity. **Part 2**: Development of a qIVIVE Model, a Case Study Using an Inhalational Drug Candidate - (AB-06)

5.3 **Chang G. Woo *(KOREATECH)*, Y-J. Cho, N.L. Jeon,** Standardization of lung Microphysiological System considering continuous flow exposure - (AB-45)

5.4 **Ji Hyun Lee *(HCT Seattle)*, M.S. Jo, H.P. Kim, Y. Han, Y.T. Kwon, K. Ahn, IL JE Yu,** Dosimetry and Dose Metric Considerations on In Vitro Inhalation Toxicity Testing - (AB-49)

10:20 am – 10:45 am Refreshment Break

10:45 am – 11:45 am **Session 6: *In Vitro***

Co-Chairs: Annie Jarabek, *(US EPA)*
Flemming Cassee, *(RIVM, Netherlands)*

6.1 **Sandro Steiner *(PMI R&D, Philip Morris Products S.A.)*, F. Lucci, A.K. Kuczaj,** InHALES: A Mechanical Replica of the Human Respiratory Tract for Advanced In Vitro Inhalation Studies - (AB-31)

6.2 **Amr Seifelnasr *(Biomedical Engineering, University of Massachusetts)*, Jinxiang Xi,** E-Vapor Enters Paranasal Sinuses: An In Vitro Evaluation Using Patient-Specific 3D-Printed Models - (AB-36), and 7.14

6.3 **Ali Doryab *(Helmholtz Munich)*, C. Gabriel, O. Schmid,** An In Vitro Fibrosis Mini-Lung with Real-Time Monitoring of Cell Mechanics for Improved Clinical Relevance - (AB-37)

11:45 am – 1:00 pm Hosted Lunch, including Student Career Conversation Breakout

1:00 pm – 3:05 pm **Session 7: *Posters***

Co-Chairs: Michael Kleinman, *(University of California, Irvine)*
Andrea De Vizcaya-Ruiz, *(University of California, Irvine)*

7.1 **Benoit Delache *(Paris-Saclay University)*, J. Creppy, T. Naninck, C. Hérate, M. Cabrera, F. Relouzat, Q. Sconosciuti, E. Navarre, F. Ducancel, R. Le Grand, L. Vecellio,** Development of a Nebulizer Device for Pathogen and Therapeutic Agents Aerosol Delivery in Cynomolgus Macaques - (AB-08)

7.2 **Jana Kesavan *(U.S. Army Combat Capabilities Development Command Chemical Biological Center)*, J. Kesavan, B.L. Laube, G. Farias, N. Karavas, M. Blondel, J. Suman,** Targeting Aerosol Delivery to Regions of Nasal-Associated Lymphoid Tissue in Three Dimensional Models of Human Intranasal Airways Using the BiVax Intranasal Atomizer - (AB-10), and 4.2

7.3 **Michael Dzierlenga *(Center for Public Health and Environmental Assessment -CPHEA)*, A. Bernstein, D. Kapraun, P. Schlosser, A. Shirke, V. Morozov,** Preliminary Modeling to Evaluate the Risk from Inhalation of Per- and Polyfluorinated Alkyl Substances (PFAS) - (AB-12), and 9.3

7.4 **Mohammad Hejazi *(Department of Mechanical and Nuclear Engineering, Virginia Commonwealth University)*, X. Owen, J. Wilkins, T. Schuman, M. Hindle, W. Longest, R. Walenga, A. Kaviratna, B. Newman, and L. Golshahi,** Understanding the Impact of Disease on Pediatric Regional Intranasal Drug Delivery with Nasal Sprays - (AB-13)

7.5 **Dominic Hoffman *(University of Delaware)*, I.R. Woodward, Y. Yu, C.A. Fromen,** Enhancing Pulmonary Drug Delivery with the TIDAL Model: A New Approach to In Vitro Aerosol Dosimetry - (AB-17)

7.6 **Jeffry D. Schroeter *(Applied Research Associates)*, G.J.M. Garcia, M. Rose, J.S. Kimbell, R.L. Walenga,** A Hybrid CFD-PBPK Approach to Simulate Deposition, Absorption, and Bioavailability of Corticosteroid Nasal Sprays - (AB-18)

7.7 **Owen Price *(Applied Research Associates, Inc.)*, B. Asgharian, S. Wasdo, R. Haskins, K. Peters, S. Chemerynski, and J. Schroeter,** Inhalation of High Vapor Pressure Constituents from Electronic Nicotine Delivery Systems - (AB-20)

7.8 **Taylor Jefferis *(Baylor University)*, D. Stevens, Y. Baldovinos, C.M. Sayes,** Exploring the Effects of Aerosol Exposure: Dose-Response Dynamics and Potential Outcomes - (AB-25)

7.9 **Christie M. Sayes *(Baylor University)*,** Exploring the Effects of Aerosol Exposure: Dose-Response Dynamics and Potential Outcomes - (AB-26)

7.10 **Yanira Balldovinos *(Baylor University)*, P. Obiako, C. Collom, T. Jefferis, C.M. Sayes,** Applying Two Alternative Exposure Methods for Toxicity Screening of E-Liquid Binary Mixtures - (AB-27)

7.11 **Francesco Lucci *(PMI R&D, Philip Morris Products S.A.)*, F. Lucci, A.R. Kolli, A.K. Kuczaj,** Evolving Aerosol Dosimetry Integrated PBPK

Modelling for Inhalation Delivery Systems - (AB-29)

7.12 **Mathew Eden *(Northeastern University)*, J. Matz, M.J. Gollner, C. Bellini, J.M. Oakes,** Smoke Dosimetry Computed with a Hybrid Lung Model in Mice Exposed to Wildland Fire Smoke - (AB-33)

7.13 **Charbel T. Yazbeck *(Northeastern University)*, M.J. Eden, J. Matz, M.J. Gollner, C. Bellini, J.M. Oakes,** Modeling and Evaluating the Pulmonary Impacts of a Wildland Firefighting Career Through Equivalent Murine Exposure - (AB-35)

7.14 **Amr Seifelnasr *(University of Massachusetts)*, J. Xi,** E-Vapor Enters Paranasal Sinuses: An In Vitro Evaluation Using Patient-Specific 3D-Printed Models - (AB-36), and 6.2

7.15 **David Chalupa *(University of Rochester)*, S. Romanick, J. McGrath, A. Elder,** Methods for Collecting and Evaluating Morphology and Quantity of Inhalable Airborne Microplastic Particulates - (AB-40)

7.16 **Matthew D. Stout *(NIH/NIEHS)*, G. Roberts, P-L. Yao, A. Gupta, J.S. Richey, J.R. Shaw, D. Fallacara, A. Wang, A. Easley, K.E. Elsass, B.J. Ellis, R.L. Ray,** Generation and Characterization of Libby Asbestos Aerosol During Chronic Inhalation Toxicology Studies - (AB-42)

7.17 **Amit Gupta *(Battelle Memorial Institute)*, B. Moyer, S. Pearson, R. Devine, J. Richey, J. Shaw, J. Newswanger, C. Griffin, D. Fallacara, W. Gwinn, P-L. Yao, G. Roberts,** Characterization of an Air Liquid Interface (ALI) Exposure System to Support New Approach Methodologies for Inhalation Toxicology Assessments Using the Flavoring Agent 2,3-Pentanedione - (AB-43)

7.18 **Shamudra Dey, *(Marquette University & Medical College of Wisconsin)*, J.M. Bock, G.J.M. Garcia,** Dry Powder Inhaler Deposition in the Larynx and the Risk of Steroid Inhaler Laryngitis: A Computational Fluid Dynamics Study - (AB-44), and 4.4

7.19 **Marion Blayac *(Helmholtz Munich)*, N. Jeliaskova, T. Stoeger, O. Schmid,** Surface Area-Based Toxicity Ranking of Aerosolized Nanomaterials from Epithelial Lung Cells Cultured Under Air-Liquid Interface Conditions: A Meta-Analysis - (AB-47)

7.20 **Martin Graffigna *(Georgia Institute of Technology)*, I.R. Bartol, M. Tano, S.A. Dewji,** Exploratory Application of DMD for Particle Deposition in the Respiratory Tract - (AB-48)

3:05 pm – 3:30 pm Refreshment Break

3:30 pm – 5:30 pm **Session 8: *Complex Aerosols***

Co-Chairs: Mark Hoover, *(Mark D Hoover LLC)*
Günter Oberdörster, *(URMC Rochester*)

8.1 **Jinho Lee *(Baylor University)*, W.C. Su,** Assessment of Respiratory Deposited Mass and Health Risks for Toxic Metals in E-cigarette Aerosol - (AB-16)

8.2 **Jeffry D. Schroeter *(Applied Research Associates Inc.)*, M. Jacketti, J. Chesnutt, B. Asgharian, O. Price, S. Wasdo, R. Haskins, K.O. Peters, S. Chemerynski,** Use of Inhalation Dosimetry Models to Evaluate the Dose Response Behavior of Electronic Nicotine Delivery Systems Aerosol Constituents - (AB-19)

8.3 **Jennifer Chesnutt *(Applied Research Associates Inc.)*, O. Price, J. Schroeter, R.M. Haskins, S. Wasdo, K.O. Peters, S. Chemerynski,** Vapor Uptake and Droplet Deposition of Tobacco Product Constituents in the Upper Respiratory Tract Using Computational Fluid Dynamics - (AB-21)

8.4 **Flemming Cassee *(RIVM, Netherlands)*,** Biodistribution and Histological Analysis of TiO2, SiC, and SiC-TiO2 and SiO2-APTES Nanoparticles in Rats - (AB-24)

8.5 **Francesco Lucci *(PMI R&D, Philip Morris Products S.A.)*, E.M.A. Frederix, S. Tajfirooz, A.K. Kuczaj,** Deposition of Evolving Multispecies Aerosol Mixtures in the Airways During Transient Inhalation Including Aerosol Uptake and Mouth-Hold - (AB-30)

8.6 **Matthew J. Eden *(Northeastern University),* H.M. Cotto, Y.M. Farra, J. Matz, C. Bellini, J.M. Oakes,** Linking Dosimetry, Pharmacokinetics, and Long-Term Health Implications in Mice Exposed to Cigarette Smoke and Pod-Mod E-cigarette - (AB-32)

5:30 pm Busses to Hotels

Friday, October 18
Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering

8:00 am – 9:00 am Hosted Breakfast

9:00 am – 10:15 am **Session 9:** ***Additional Applications***

Co-Chairs: **Annie Jarabek,** ***(US EPA)***
Otmar Schmid, ***(Helmholtz Munich)***

9.1 **Benoit Delache** ***(aParis-Saclay University)*****, C. Herate, A.-R. Garnier, O. Gorgé, J.N. Tournier, N. Verguet, F. Relouzat, J. Creppy, Q. Sconosciuti, E. Navarre, V. Contreras, M. Cabrera, R. Le Grand, F. Ducancel , L. Vecellio, T. Naninck,** Comparison of aerosol exposure systems for pathogen delivery in non-human primates using PET-CT imaging - (AB-07)

9.2 **Bahman Asgharian** ***(Applied Research Associates, Inc.)*****, O.T. Price., J. Schroeter, J. Rodriguez,** Drug Delivery to the Lungs of Humans and Rats to Protect Against High Altitude Pulmonary Edema (HAPE) - (AB-09)

9.3 **Michael Dzierlenga** ***(Center for Public Health and Environmental Assessment (CPHEA))*****, A. Bernstein, D. Kapraun, P. Schlosser, A. Shirke, V. Morozov,** Preliminary Modeling to Evaluate the Risk from Inhalation of Per- and Polyfluorinated Alkyl Substances (PFAS) - (AB-12), and 7.3

9.4 **Emmanuel Mate-Kole** ***(Georgia Institute of Technology)*****, S.A. Dewji,** Stochastic Modeling of Radionuclide Inhalation Dosimetry for Optimized Radiation Countermeasures and Consequence Management Applications - (AB-51)

10:15 am – 10:40 am Refreshment Break

10:40 am – 12:20 pm **Session 10:** ***Discussion and Research Needs***

Co-Chairs: **Chantal Darquenne,** ***(University of California, San Diego)***
Robert Phalen, ***(University of California, Irvine)***

12:20 pm - 12:30 pm Instructions for Authors, Conference Evaluation, & Research Needs Form

12:30 pm Busses to Hotel

III. ABSTRACTS

AB-01

Modeling Aerosol Bolus Dispersion and Deposition in the Human Lung

Bahman Asgharian[1]*, Owen Price[1], Azadeh A.T. Borojeni[2], Andrew P. Kuprat[3], Rajesh Singh[3], Richard A. Corley[4], Chantal Darquenne[2].

[1]Applied Research Associates, Inc., Arlington Division, Raleigh, NC, USA, [2]Department of Medicine, University of California, San Diego, CA, USA, [3]Pacific Northwest National Laboratory, Richland, WA, USA and [4]Greek Creek Toxicokinetics Consulting, LLC, Boise, ID, USA.

Inhaled particles undergo particle mixing by different physical mechanisms in various regions of the lung. Mixing is a higher order effect, which cannot be directly captured in a one-dimensional (1D) modeling approach. Existing 1D particle deposition models either ignore the phenomenon or model it as an apparent diffusive term to allow for rapid computation of particle deposition. Reported apparent diffusivities by different investigators were based on particle mean velocity and airway diameter and were obtained from particle concentration measurements from in-silico and in-vitro studies in tracheobronchial (TB) airways. No in-vivo data so far has been used to develop a convection-based mixing model in the alveolar region.

We developed a mixing model for an inhaled aerosol bolus traveling through airways of the human lung. Mixing in the extrathoracic region was determined based on computational fluid dynamics simulations in a realistic model of the oral airway. The aerosol concentration profile exiting the oral airway was used as input in the 1D Multiple Path Particle Deposition (MPPD) model used to simulate transport and deposition in the intrathoracic region of the lung. The MPPD model was modified to include the widely used apparent diffusivity coefficient to account for mixing in the TB airways. In addition, we revised the transport model in the pulmonary airways by separating respiratory airways and alveoli and developing transport models across these airways. A constant coefficient was introduced to account for the alveolar mixing, which was determined by comparing model predictions of aerosol bolus deposition, dispersion, and mode shift with measurements of Darquenne et al. (J. Aerosol Sci, 2016). Good agreements were observed between predictions and measurements. In conclusion, the combined dispersion-deposition MPPD improves predictions of aerosol deposition and dispersion in the human lung, providing an updated tool for exposure risk assessment and targeted drug delivery. *Funding: NIH/NIEHS 1U01ES028669.*

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Updating Aerosol Inhalability (4/3/2024)

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For inhaled aerosol particles to have health effects, entry into the nasal or oral openings and then deposition on an airway surface, are necessary. The inhaled entry efficiency for an aerosol particle, a function of its aerodynamic diameter, is referred to as its inhalability, inspirability, or sampling efficiency. Understanding the aerosol entry efficiency is important in interpreting epidemiological, clinical, and in-vitro studies involving the effects of inhaled particulate materials. The widely accepted inhalability functions published by the American Conference of Governmental Industrial Hygienists (ACGIH) and other organizations have been important for setting recommended workplace air quality concentrations, such as Threshold Limit Values. The ACGIH deposition efficiency function decreases from 100% for the smallest particles to 50% for 100 µm (aerodynamic) diameter particles. It is applicable to wind speeds from 0.4 to 4 meters/s, and represents the aerosol inhalability for a vertical adult male averaged over 360 degrees yaw angle to the wind. For other applications, the inhalability might need to be corrected if wind speeds are outside of the ACGIH range, if there are large differences in body-surface and ambient temperatures, or there are other relevant factors. This is especially true for particles with aerodynamic diameters above about 10 µm and for wind speeds above about 10 m/ s. Wind tunnel and modeling research has provided inhability equations applicable to many exposure scenarios, but cases involving highly-charged aerosol particles, or young children are poorly understood. Other factors that require more research, include body motion and posture, facial hair, and pitch and roll angles of the head relative to the wind.

Unfinished Business in Particle Inhalation Dosimetry

Günter Oberdörster and Uschi Graham
URMC Rochester

It was Paracelsus - about 500 years ago - who combined chemistry and biology to become the foundation of today's Toxicology. He also emphasized the importance of Dose to express the toxicity of a substance. This basic principle of toxicology often appears to be forgotten when in the search for molecular mechanisms of toxicity extraordinarily high doses are used, so that Paracelsus' principle may have to be paraphrased: "The Dose not only makes the poison, but the dose also makes the mechanism". The accurate measurement of dose, defined as dosimetry, is a focal point of inhalation toxicology of particles but also involves matters of dose-metric, dose-rate, micro-dosimetry, in vitro-dosimetry, translational dosimetry. Whereas the expanding field of dosimetry has produced a number of scholarly written publications, many of them have just scratched the surface, awaiting subsequent in-depth research, representing "unfinished business" some of which is summarized in the following.

One practical dosimetry associated application relates to risk extrapolation involving the four steps of risk assessment, requiring detailed dosimetric translation *(e.g. lab animal to human)*. Another example pertains to our accidental finding 30 years ago of the increased biological activity of poorly soluble ultrafine particles compared to the same mass of chemically alike fine particles. This particle size dependent difference in biological/toxicological activity raised the question of dose-metrics: Is the conveniently applied metric of particle mass appropriate, or should other metrics be considered? Also to be considered is the impact of particle solubility *(static)* and dissolution *(dynamic)* affecting pulmonary particle clearance and retention kinetics

In vitro studies delivering aerosols to cells or respiratory tract tissues need to establish the relevancy to in vivo conditions with respect to dose equivalency between the two exposure modes. Essential is the verification of dose per unit target cell/tissue. As a caveat, even with the establishment of a sound dosimetric in vitro/in vivo model, the fact that in vitro cell and tissue cultures have been separated from their physiological milieu may make them prone to responses in vitro that are different from in vivo.

A long recognized yet often overlooked or neglected problem in particle inhalation dosimetry is the failure of using the appropriate density of inhaled agglomerates, specifically of ultrafine particles.. Using the material density of agglomerated or aggregated aerosols of nanoparticles as input into a particle deposition model disregards the many void spaces in between the accumulated particles. Determining instead the much lower effective density "in vivo" in a rat inhalation study is possible by using the downloadable yet uncorrected version 3.04 of the MPPD model.

Suggestions for future developments of particle inhalation dosimetry models include modeling of particle translocation rates from the respiratory tract as portal of entry to secondary organs: An example is the translocation of inhaled nanosized pollution particles in humans via neuronal pathways from the nose to the olfactory bulb in the brain. Using advanced high resolution analytical imaging (STEM/EELS) of brain tissues led to amazing, pioneering findings. These, including dosimetry suggestions, will be presented separately in the next session.

The above few examples of ongoing particle inhalation dosimetry studies illustrate their importance for establishing Exposure – Dose – Response relationships and provide essential contributions to the medical sciences. Importantly, to successfully investigate and answer new and follow-up dosimetry related questions, close multidisciplinary teamwork is critical.

Presentation Title: *Challenges and progress in characterizing in vitro dosimetry for air-liquid interface exposures*

Jessica Murray
US EPA

Abstract:
Inhalation is one of the three primary modes of chemical exposure, but key challenges in developing reproducible exposure systems with robust analytical dosimetry methods have limited the use of *in vitro* models for the assessment of inhalable chemicals. *In vitro* inhalation assays have been improved with air-liquid interface (ALI) exposure systems which allow direct cell-toxicant interactions, but these systems are complex and vary in their geometry, flow rates, and operational parameters. Physicochemical properties of the inhaled substance must be carefully considered when selecting an ALI exposure system, optimizing its operational parameters, and developing methods to characterize exposure conditions. For example, volatile organic compounds (VOCs) and aerosols possess distinct transport and deposition mechanisms which complicate the reliability and reproducibility of deploying ALI exposure systems to screen diverse inhalable compounds without quantitative methods to assess exposures. Detection methods to quantify deposition are often missing in ALI studies, but when deposition is recorded, it is usually with a cell-free collection method (QCM, filter, etc). As an alternative approach, we aerosolized two fluorescent tracers (sodium fluorescein and rhodamine 6G) and developed methods to directly quantify deposition and cellular uptake on human bronchial epithelial cells grown at the ALI across multiple dynamic and static exposure systems, including the EPA ACCES, MedTec CelTox, and the VITROCELL Cloud α12. These fluorescent tracers revealed that particle deposition is highly variable within and across exposure systems, and cell-free collection methods did not reliably estimate cell deposition in many scenarios. Both tracers were also able to highlight differences in internal dose rates across different exposure methods and cell models utilized. Mucus retention decreased cellular uptake in differentiated primary human bronchial epithelial cultures (HBECs) compared to an immortalized cell line, indicating that further work is needed to determine how cell model selection may influence dosimetry. Overall, these results highlight opportunities to better characterize *in vitro* exposures and improve dosimetry methods for *in vitro* to *in vivo* extrapolation. [Abstract does not reflect views or policies of the U.S. EPA.]

Dosimetry concepts for in vitro prediction of respiratory toxicity.

Part 1: Identification of relevant dose-metrics, case study using a 2-stroke engine exhaust.

Ritter, D., Schwarz, K., Koch, W.

Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

An ongoing discussion exists on the relevant dose-metric regarding the toxicity of inhaled particles. Deposited particle surface versus number or mass are under discussion for different types of aerosols containing poorly soluble particles, originating from different sources of combustion, or consisting of droplets.

An experimental model was developed to study the toxicological properties of a combustion aerosol allowing for the variation of particle size, mass concentration, and number concentration. This model provided a valuable opportunity to test the most relevant dose metric.

The experimental setup involved a highly concentrated ultrafine particle aerosol (2-stroke engine exhaust). An ageing unit based on Brownian coagulation was used to independently vary number and mass concentration of the aerosol. Acute local biological effects were studied using an optimized exposure device (P.R.I.T.® ExpoCube®). Air-liquid- interface cultures (ALI) from a human lung cell line (A549) were applied, and measurements including viability (WST-1), mitochondrial membrane potential (JC-1), and Interleukin-8 release were taken.

Four different conditions represented various number concentrations and mean particle sizes while maintaining comparable aerosol mass concentrations. Dose-response relationships were established by variation of exposure time (30 or 60 minutes). Analytical validation confirmed particle deposition, and experiments with filtered aerosol demonstrated discriminating effects by particles and gas phase. The results clearly showed a strong correlation between particle mass dose and biological effect, rather than particle number dose. These findings are likely due to the physico-chemical characteristics of the aerosol particles (oily) and align well with the ongoing discussion regarding dose metrics for other types of aerosols.

The results provide valuable insights into inhalation dosimetry using the in vitro inhalation model. Such information can help identify suitable dose metrics for other aerosols, guide future research in this area and is important for predicting respiratory toxicity using in vitro to in vivo extrapolation (qIVIVE) models.

Dosimetry concepts for in vitro prediction of respiratory toxicity.

Part 2: Development of a qIVIVE model, a case study using an inhalational drug candidate

Schwarz K, Winterberg D, Obernolte H, Wronski S, Hansen T, Ritter D, Sewald K, Braun A Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

In contrast to the derivation of threshold values from in vivo data, there are no generally applicable or accepted concepts to relate dosing and cellular effects to in-vivo exposure and adverse outcomes on a quantitative basis.

The aim was to develop a quantitative approach based on various in-vitro and ex vivo methods to allow an early feasibility assessment of inhaled therapeutics using the example of nafamostat.

Local tolerability derived from cell-based inhalation assay and the rat ex-vivo isolated perfused lung (IPL) was combined with the Precision Cut Lung Slice (PCLS) model as an ex-vivo organotypic infection model to derive the local therapeutic window. A classical GLP 28-day regulatory inhalation toxicological study was performed in rats in parallel.

Dose-response relationships derived in air-liquid exposed A549-cells reveal a NOAEL of 1.5 µg/cm^2 respectively a lung deposited dose of 0.33 mg/kgbw in the whole-organ model of the isolated perfused lung. Efficacy has been shown for 0.5 µM in PCLS. Animals showed adverse effects for a 0.5 mg/kgbw dose in the larynx and upper airways (ulcer, necrosis).

To extrapolate the findings from the in-vitro and ex-vivo models to an in-vivo context and to convert the data into a standardized dose metric, the MPPD model and pharmaco- kinetic considerations for the respiratory tract were utilized. Analysis of the surface- specific dose throughout the respiratory tract in vivo and in IPLs in combination with the local pathological findings indicate at similar NOAELs of ~ 1 µg/cm^2 (in vivo) resp. 2 µg/cm^2 (IPL), which is in good agreement with the cell-based in-vitro results.

The combination of suitable in vitro and ex vivo models in combination with customized dosimetric considerations can provide promising quantitative in vitro-to-in-vivo correlations for respiratory toxicity for local (sub)acute effects of inhaled substances. This supports application of in-vitro models and guides future research in this area.

Comparison of aerosol exposure systems for pathogen delivery in non-human primates using PET-CT imaging

Authors
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Keywords
Respiratory tract deposition, aerobiology, Non-Human Primate (NHP), nuclear imaging, pathogen delivery.

Transmission of respiratory pathogens constitute a major public health concern. Non-Human Primates (NHPs) are relevant animal of human respiratory infections because of their close physiopathology, their susceptibility to pathogens affecting humans and their anatomy.

Objective: In order to compare different classical exposure systems used in aerobiology to mimic human infections we designed deposition studies in NHPs based on using liquid endotracheal instillation, mesh-nebulization adapted on facemask or head only exposure systems.

Method: PET-CT imaging was used to assess and quantify fluodeoxyglucose ([^{18}F]-FDG) deposition after inhalation through the different systems on anesthetized cynomolgus macaques. Upper respiratory tract (URT), lower respiratory tract (LRT) and oesophagus/stomach deposition were calculated according to the dose loaded in the device.

Results: We measured 99%+/-SD (0.8%), 36.6%+/-SD (3.7%) and 5%+/-SD (1.8%) of the dose deposited in total airways for instillation, facemask and head only exposure, respectively. A 100% dose was deposited in the LRT when using instillation whereas 10%+/- SD (2.2%) was deposited in the LRT with the facemask and 19%+/-SD (6%) for head only systems. In addition, radioactivity distribution homogeneity in the lung is improved when using facemask and head only exposure device.

To confirm the relevance of [^{18}F]-FDG as a relevant estimation of pathogen distribution we successfully compared *Bacillus atropheaus* spores to [^{18}F]-FDG particle size distribution after nebulization using the facemask (MMAD respectively 3.6+-0.3µm vs 3.1+-0.2µm). After inhalation by NHPs of spores mixed with [^{18}F]-FDG, similar results were obtained between radioactivity and pathogens quantity sampled in different biological fluids of respiratory tract (nasal and tracheal swabs, broncho-alveolar lavages).

Conclusion: Pathogen respiratory delivery systems influence deposited dose and distributions in NHPs airways. PET–CT imaging represents a potent tool to accurately estimate exposure doses to respiratory pathogens.

Development of a nebulizer device for pathogen and therapeutic agents aerosol delivery in cynomolgus macaques

Authors

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Keywords

Respiratory tract deposition, Non-Human Primate, Mesh nebulization, nuclear imaging, aerosol therapy, facemask.

Cynomolgus macaques (CM) has been identified as a relevant animal species for modelling human infectious respiratory diseases thanks to their anatomical similarity including cellular entry receptors for viruses. However, administration of pathogens and therapeutics via inhalation route can be challenging in terms of deposition efficacy, biosafety and reproducibility. Our objective was to develop and assess a nebulizer system allowing either pathogen or drug delivery to CM for rapid transfer to clinical trials. We then developed a bolus nebulizer system for pathogen inhalation. An Aeroneb mesh nebulizer with 0.1 mL/min liquid flow and 3.6 µm of MMAD has been connected to a customized inhalation chamber adapted to an airtight facemask prototype. The nebulizer performance has been characterized by calculating airways deposition using radioactivity on anaesthetized CM. A lung deposition of 19 ± 7% of the dose charged in the nebuliser was obtained by gamma camera imaging. A lung deposition of 16 ± 3% of the dose charged has been measured by PET-CT imaging. Aerosol deposition on animal face remains low: 0.4% of nebulizer charge. Aeroneb mesh nebulizer has been evaluated with *Bacillus atropheaus* spores as a model of pathogen. Aerosol was collected and spore's viability forming bacteria were measured. Mesh nebulizer was associated to 100% spore viability. The device has also been used for aerosoltherapy. A biological drug, IgG anti-SARS-CoV-2, has been nebulized with mesh nebulizer on five CM. We demonstrated antibody integrity and effective distribution of the antibody in the upper and lower respiratory tract.

In conclusion, we have developed a device that allows for the inhalation of pathogens or therapeutic agents with very high efficiency in terms of deposited dose while maintaining the viability or therapeutic properties of fragile molecules. This prototype is perfectly suited for the exposure of non-human primates in a classified environment for microbiological risk.

Drug Delivery to the Lungs of Humans and Rats to Protect Against High Altitude Pulmonary Edema (HAPE).

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High-altitude pulmonary edema (HAPE) may occur within hours following rapid transport to high elevations. Medical treatment by inhalation generally requires delivery of the drug to the target sites within the lung. However, optimal size distribution and concentration of the aerosolized drug is needed to target affected sites within the pulmonary region. We utilized mathematical modeling to aid in drug formulation and delivery by determining the fate of inhaled drugs in HAPE patients. Modeling efforts included construction of realistic lung geometries in humans and laboratory animals, drug formulation based on physicochemical properties of the compound, and development of a mathematical model to study transport and deposition of aerosolized drug particles in the lungs. We developed realistic, asymmetric lung geometries in Sprague Dawley (SD) rats and humans based on available morphometry and scanned imagery. Existing reconstructed lung geometries artificially separate the conducting and respiratory zones to allow for fast computation of the deposited drugs. We allowed each lung pathway to reach the terminal bronchiole and alveolar space at a different depth, thereby creating anatomically accurate, multiple path lung geometries in SD rats and humans. In addition, we developed a mathematical model for transport and deposition of inhaled drug particles to predict the dose to target sites of the lung as a function of drug particle size. Model predictions showed that at normal breathing only 7% of generated particles reached and deposited in the pulmonary regions of the rat lung, which were the target sites for drug delivery. The peak deposition occurred for particles near 2 micrometers in rats. In humans and via oral breathing, about 15% of particles deposited in the pulmonary region for particle sizes around 4 μm. This study was funded by Medical Technology Enterprise Consortium (MTEC) number 2018-679.

Targeting Aerosol Delivery to Regions of Nasal-Associated Lymphoid Tissue in Three Dimensional Models of Human Intranasal Airways Using the BiVax Intranasal Atomizer

Jana Kesavan, Beth L. Laube, Goncalo Farias, Nektaria Karavas, Mathilde Blondel, Julie Suman

Well-organized nasal-associated lymphoid tissue (NALT) is found in the pharyngeal and tubal tonsils of adults and children and diffuse NALT is found in the superior, middle and inferior turbinates of young children. However, it is not clear how to target these sites with aerosolized vaccines. We explored three head positions (upright [Up], tilted back at 45° [45] and supine [Su]), two angles of insertion (30° and 45°) and two distances of insertion (6mm and 9mm) as ways to direct aerosol to NALT sites in three-dimensional models of the intranasal airways of an 18 and a 5 year old (yo). Fluorescein aerosol was generated by an Aptar Pharma BiVax 200 µL intranasal atomizer. Percent fluorescein deposition was quantified in the anterior nose, superior and middle turbinate regions, inferior turbinate/nasopharynx, and on an exit filter. Mean percent deposition in both models was <0.5% in the superior turbinate and the exit filter for all test conditions. Multivariate analyses showed that deposition was unchanged by the angles and distances of insertion. Middle turbinate deposition was significantly higher in the 5 yo than in the 18 yo ($p=0.01$) and was Up<45<Su for both models ($p<0.01$). Inferior/nasopharynx deposition was Up>45>Su for both models ($p=0.03$) and anterior nose deposition was higher in the 18 yo than in the 5 yo ($p<0.01$). These results suggest: (1) supine and upright head positions might be used to target delivery of aerosolized vaccines generated by the BiVax intranasal atomizer to NALT sites in the middle turbinate and inferior turbinate/nasopharynx, respectively, in patients ≥5 years of age; and (2) delivery to the middle turbinate may be higher in children ≤5 yo and deposition in the anterior nose may be higher in adults, for all head positions. *In vivo* tests are needed to confirm these findings.

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Validation of computational fluid dynamics simulations of nasal sprays with regional doses measured by gamma scintigraphy

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Objectives: Nasal sprays are widely used to deliver pharmaceutical aerosols to the nasal passages. Computational fluid dynamics (CFD) can estimate the regional doses delivered by nasal sprays, but few studies have validated CFD predictions of regional deposition with experimental data. This study aimed to compare the regional doses estimated by CFD versus in vitro experiments with a novel gamma scintigraphy technique.

Methods: Anatomically accurate models of 6 adult nasal cavities (n=12 unilateral cavities) were reconstructed from computed tomography scans. Nasal replicas were fabricated with a soft anterior nose and a rigid posterior nose connected at the nasal valve. Gamma scintigraphy experiments quantified the intranasal distribution of Nasacort Allergy 24HR (triamcinolone acetonide nasal metered spray) at a constant unilateral flowrate of 7.5 L/min. CFD simulations were performed in ANSYS Fluent assuming a spray cone angle of 55.9°, spray velocity of 7.1 m/s, and a log-normal particle size distribution (PSD) with mass median diameter of 43.8 μm and span of 1.99. The nozzle position was estimated from the gamma scintigraphy images. Statistical significance was tested with a paired Student's t test at the level $p<0.05$.

Results: The nasal spray dose that reached the posterior nose was smaller in the simulations than in the experiments (37±9% vs. 46±15%, $p=0.003$). A systematic parameter sensitivity analysis was performed to investigate how posterior dose was influenced by changes in spray cone angle, insertion depth, spray velocity, and PSD, but none of these factors explained the smaller posterior dose in the simulations.

Conclusions: Gamma scintigraphy and CFD simulations agreed that a significant fraction of the nasal spray deposited in the nasal vestibule, but a statistically significant difference was observed between experiments and simulations. Future studies should investigate if the agreement can be improved using a wall-film boundary condition that allows droplets to splash and rebound from walls.

Preliminary Modeling to Evaluate the Risk from Inhalation of Per- and Polyfluorinated Alkyl Substances (PFAS)

Michael Dzierlenga, Amanda Bernstein, Dustin Kapraun, Paul Schlosser, Avanti Shirke, Viktor Morozov

Center for Public Health and Environmental Assessment (CPHEA), Office of Research and Development (ORD), U.S. EPA

While much research has focused on the toxicity from ingestion of per- and polyfluorinated alkyl substances (PFAS), their presence in indoor and outdoor air raises concern about the risks posed by inhalation exposure. To evaluate the feasibility of extrapolating ingestion risk metrics to inhalation, we performed a systematic review to identify studies reporting PFAS inhalation administration with the potential to inform pharmacokinetic modeling. This resulted in the identification of 22 studies with human evidence and 30 studies with animal evidence. We then examined a PFOA inhalation study as a test-case to understand how easily a physiologically based pharmacokinetic (PBPK) model designed for ingestion could be adapted for inhalation. We used the Multipath Particle Dosimetry (MPPD) model to predict an overall deposition fraction of 44% in rats, with 65% of the deposition occurring in the head. Smaller fractions were deposited in the tracheobronchial region (29%) and the alveolar/pulmonary region (5%). With the inhaled dose treated as an ingestion, the PBPK model performed well at the higher doses of 10 mg/m^3 and 25 mg/m^3, with a predicted:observed average plasma concentration ratio of 1.0 and 1.1 respectively. However, for the lowest dose of 1 mg/m^3, the model underpredicted the data substantially, with a predicted:observed average plasma concentration ratio of 0.35. Overall, while the model performance was better than expected, further model development is needed to capture the nonlinear kinetics displayed in the observed inhalation data.

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

Understanding the Impact of Disease on Pediatric Regional Intranasal Drug Delivery with Nasal Sprays

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Currently, there is uncertainty with pediatric intranasal drug delivery and the impact of disease on intranasal deposition patterns - following nasal spray administration. We have recently studied intranasal drug delivery of suspension nasal sprays in children 2-11 years old (50% <6 years old and 50% female) with healthy nasal airways [1]. In this study, 8 realistic CT-based diseased nasal cavities of 4 children 5-10 years were prototyped and used to study the *in vitro* regional drug deposition of Nasacort ALLERGY 24HR nasal spray (55 mcg triamcinolone acetonide per spray), and compare the deposition pattern with the previously-developed representative healthy nasal models [1]. To do so, models were mounted on top of the nasal spray and the outlets of the models were connected to a breathing simulator, generating a “gentle sniffing” pattern. At the onset of inhalation, the spray was actuated by an automatic actuator. Subsequently, the models were dismantled, and the drug in each piece was recovered with an appropriate solvent. The samples were assayed to determine the drug concentration using high-performance liquid chromatography (HPLC). The amount of drug deposited in the two sides of the nasal airway with significant nasal septum deviation (NSD) was significantly different in all of the nasal cavity regions. Significant differences between the two sides were also observed in at least one region of the nasal cavity for one subject with significant airway edema, chronic rhinosinusitis (CRS), and minor NSD. No significant differences were obtained for the two other subjects, one with significant hypertrophy in the left side, and another with severe CRS. Comparing the results with the healthy models showed that the observed range of drug deposition in the turbinate region of healthy models is not significantly different from the diseased models, while it is significantly different in the proximal turbinate and the vestibules.

Reference:

[1] Esmaeili, A. R., Wilkins, J. V., Hosseini, S., Alfaifi, A., Hejazi, M., Hindle, M., ... & Golshahi, L. (2024). In vitro evaluation of intersubject variability in pediatric intranasal drug delivery using nasal spray suspension products. *Journal of Aerosol Science*, 179, 106387.

Anatomically-Realistic Adult and Pediatric Nasal Models as Platform Tools to Study In Vitro Regional Drug Delivery and Bioequivalence of Suspension Nasal Sprays

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Abstract
In vitro studies, including droplet size distribution and plume geometry measurements, are recommended to establish bioequivalence (BE) between a potential generic nasal product and its reference listed drug (RLD) for suspension formulations of locally acting nasal drug products by regulatory agencies. While these in vitro BE studies can be more sensitive to detect performance differences, compared to in vivo comparative clinical endpoint BE studies, their clinical relevance may be limited as compared to other potential in vitro performance metrics. For example, in vitro regional nasal deposition may provide a performance metric that can measure the fraction of active pharmaceutical ingredient (API) reaching the target region directly. The target region for many locally acting nasal sprays is generally defined as the region beyond the internal nasal valve (INV), but prior to the nasopharynx. Our team performed a study in collaboration with a clinical head and neck surgeon to define the plane of INV in digital 3D nasal airway geometries [1]. Furthermore, differences in nasal anatomy can further complicate evaluating regional nasal deposition using standard in vitro methods, which currently do not account for nasal anatomy and the interaction of the nasal drug products with the complex anatomy of nasal airways. The goal for this presentation is to discuss the recent development of in vitro anatomical nasal models of adult [2,3] and pediatric [4] human subjects, the range of drug delivery to the target regions of nasal airways in both age groups [2,4], and the sensitivity of these models in detecting performance differences when utilizing suspension-based nasal spray products. Subsequent population bioequivalence (PBE) analyses of the regional nasal deposition in six representative realistic airway models of three healthy adult and three healthy pediatric subjects suggested that the anatomical differences among subjects may impact the nasal spray performance across different nasal products.

References
[1] Computers in Biology and Medicine, 2020, 123, 103896. [2] International Journal of Pharmaceutics, 2021, 593, 120103. [3] International Journal of Pharmaceutics, 2022, 622, 121858. [4] Journal of Aerosol Science, 2024, 179, 106387.

Monte-Carlo simulations of stochastic asymmetric bronchial trees: Towards targeted pulmonary drug delivery

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1 Abstract

Pulmonary drug delivery has emerged as a preferred route of drug administration due to its benefits like high effectiveness and lesser side effects as compared to other methods. Inhaled drugs can be categorized into two classes: those targeting the airway tissue itself, absorbed in the upper lung, and those intended to reach the deep lung and be absorbed into the bloodstream for systemic effects. Therefore, understanding and predicting regional deposition patterns of inhaled drugs becomes crucial for devising drug delivery strategies. Various factors such as particle size, inhalation rate, etc. can influence the deposition of inhaled drugs in different regions of the respiratory tract. Most importantly, the trajectories of inhaled particles depend on the unique geometry of each person's respiratory tract. Thus, variation in lung anatomy is a major reason why different people respond to inhaled medications differently.

To that end, we have developed a stochastic asymmetric Multi-Path Particle Dosimetry (MPPD) model of the human airways. The tracheobronchial airways were generated based on Hess-Murray's law and regular asymmetric branching. Symmetric and alveolated acinar sub-trees were attached to the terminal bronchioles. We introduced stochasticity in the branching asymmetry around its mean values to generate biologically realistic and representative models of the lung geometry. Aerosol deposition was calculated using a combination of well- established analytical and empirical relations. Through Monte-Carlo simulations for several realizations (n = 6000) of lung geometries, inter-subject variability in regional particle deposition in large diverse populations was quantified. We present the statistical distributions of regional deposition while varying several key parameters - branching asymmetry (r), particle size (d_p), breathing period (T_b), bronchoconstriction (β), etc. These insights are valuable for determining drug dosages as well as design and choice of delivery devices (inhalers/nebulizers).

Assessment of Respiratory Deposited Mass and Health Risks for Toxic Metals in E-cigarette Aerosol

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Inhalation and deposition of e-cigarette aerosol in the human respiratory tract can lead to various adverse health effects. Therefore, studying the respiratory deposition of e-cigarette aerosol is crucial to assess the deposited mass of specific toxic substances in the human airways, allowing a more accurate estimation of associated health risks. This research developed and applied a novel aerosol respiratory deposition method, the MALDA-MOUDI approach, which integrates the Mobile Aerosol Lung Deposition Apparatus (MALDA) and Micro-Orifice Uniform Deposit Impactors (MOUDI) to study e- cigarette aerosol respiratory deposition. MALDA includes a set of 3D-printed realistic human airway replicas that encompass the human head airways (with oral and nasal cavities), the tracheobronchial tree (down to the 11th bifurcation), and the alveolar region (constructed with porous foam). MOUDI is a cascade aerosol sampler. A series of laboratory experiments were conducted to investigate e-cigarette aerosol respiratory deposition via both active and passive vaping. Three types of e-cigarettes: JUUL, Disposable, and IQOS were used in this research, with a specific focus on the toxic metals present in the aerosol. The size-dependent deposited masses of e-cigarette aerosol in different airway regions were systematically investigated using the MALDA-MOUDI approach. Potential health risks posed by metal deposition from e-cigarette aerosol in the airways were carefully assessed based on reasonable assumptions regarding active and passive vaping scenarios. Results obtained indicated that the metal- induced non-cancer risks were generally acceptable, with estimated hazard quotients and hazard index all below 1.0. Additionally, calculated metal-induced cancer risks were found to be within acceptable limits. The lifetime excess cancer risks were all less than 10^{-6}. Based on these findings, inhalation of e-cigarette aerosol does not appear to pose significant metal-related respiratory health risks.

Title:
Enhancing Pulmonary Drug Delivery with the TIDAL Model: A New Approach to In Vitro Aerosol Dosimetry

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Format Preference: Poster

Abstract:
Understanding where inhaled aerosol drugs are deposited within the lungs is essential to optimizing their efficacy and reducing off target effects. In this regard, experimental *in vitro* deposition assessments are a critical component of the preclinical pipeline, serving to validate computational simulations and provide spatial information in the absence of clinical studies. However, most existing *in vitro* models fail to capture the complexities of lower lung generations, perform full inhalation-exhalation breathing maneuvers, or assess an entire inhaled dosage. To address this gap in existing *in vitro* approaches, our lab has recently developed a tool we coined TIDAL-Total Inhalable Deposition in an Actuated Lung model. The TIDAL platform consists of 3D-printed patient-derived upper airways connected to deformable lobe units containing internal lattice structures that mimic the lung surface area and free volume to provide spatial deposition mimicry. The modular design allows for high-resolution deposition data and customizability to varied patient populations. Our initial TIDAL prototype of a healthy male has a demonstrated total lung volume over 7 liters and breathing rates over 30 LPM. Adopting a resting, symmetric breathing profile exchanging ~600 mL of air at a peak inspiratory flow rate of 21.5 ± 1.0 LPM, TIDAL was able to generate spatial deposition maps of fluorescence aerosols nebulized from an Aerogen Lab Nebulizer (MMAD 6.77 μm) with a planar-equivalent central to peripheral ratio (C/P) of 1.08 ± 0.03. This C/P result is in line with analogous human scintigraphy studies, supporting the relevance of our design in effectively generating accurate spatial deposition profiles. Building from these promising results, our on-going efforts include updating the range of breathing profiles and validating the model using a broader range of aerosol sizes to increase the utility of this new *in vitro* platform.

A Hybrid CFD-PBPK Approach to Simulate Deposition, Absorption, and Bioavailability of Corticosteroid Nasal Sprays

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Corticosteroids delivered by aqueous suspension nasal sprays are commonly used to treat allergic rhinitis. Corticosteroid absorption is influenced by many factors such as regional nasal deposition, dissolution rates, and physicochemical properties of the drug. Computational fluid dynamics (CFD) simulations were used to estimate regional droplet deposition from nasal sprays in healthy and rhinitic models that were partitioned into different anatomical regions. A whole-body physiologically based pharmacokinetic (PBPK) model was developed to simulate the drug absorption and bioavailability. Anatomical regions from the CFD models were included as compartments in the PBPK model to assess effects of regional dosimetry of nasal sprays. The nasal compartments included mucus, epithelium, and submucosal sub-compartments to simulate mucociliary clearance, dissolution, and epithelial permeability. Model simulations were conducted for three commercial steroid nasal sprays: fluticasone propionate (FP), mometasone furoate (MF), and budesonide (Bd). PBPK parameter fitting and model verification were conducted using experimental data from studies in the literature for oral, intravenous, and intranasal dosing. Predicted nasal epithelial and plasma concentrations varied widely depending on drug solubility (0.1, 0.14, and 16.0 μg/mL for MF, FP, and Bd) despite similar nasal deposition patterns. At a 100 μg nasal spray dose, maximum plasma concentrations were 8.9 pg/mL and 7.0 pg/mL for the low-solubility drugs FP and MF, respectively, whereas predicted maximum plasma concentration for the high-solubility Bd was 717.7 pg/mL. The CFD-PBPK approach can be used to examine pharmacokinetic differences between steroids and assess effects of regional nasal deposition on local nasal tissue and systemic kinetics.

Use of Inhalation Dosimetry Models to Evaluate the Dose Response Behavior of Electronic Nicotine Delivery Systems Aerosol Constituents

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Inhaled aerosols emitted from electronic nicotine delivery systems (ENDS) contain nicotine, propylene glycol, glycerin, flavors, and other harmful and potentially harmful constituents (HPHCs). While inhaled HPHC concentrations from ENDS are typically less than mainstream smoke from combustible cigarettes, knowledge of inhaled aerosol dose-response behavior is needed to evaluate potential toxicity. We developed a dosimetry model that accounted for physicochemical properties of ENDS aerosol constituents and included thermodynamic mechanisms (i.e., phase change, coagulation, heat exchange, and nicotine protonation) to predict droplet deposition and vapor uptake of constituents in the respiratory tract. The dosimetry model was used to predict daily lung dose of each constituent based on user topography (e.g., puff duration and mouth-hold time) and daily usage regimen (e.g., puffs/session and sessions/day). Exposure-response data was compiled from available inhalation toxicity studies on individual ENDS constituents. Computational fluid dynamics in the human and rat upper respiratory tracts and mechanistic models of lung dosimetry were used to predict regional respiratory tract dose of each constituent and to predict the daily dose values for exposure scenarios from published studies. Daily dose predictions from ENDS simulations were calculated for several HPHCs, including formaldehyde, acetaldehyde, acrolein, benzaldehyde, and 1,2-pentanedione. Predicted dose values were compared to regional respiratory tract dose predictions derived from individual exposure scenarios in rats and humans in which a range of respiratory effects, such as non-cancer and cancer outcomes, were observed. The results demonstrate the potential use of dosimetry models for evaluating hazards and health risks of complex multi-constituent aerosols based on internal dose. This study was funded by the Center for Tobacco Products at the U. S. Food and Drug Administration. This is not a formal dissemination of information by FDA and does not represent Agency position or policy.

Inhalation of high vapor pressure constituents from electronic nicotine delivery systems

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Disclaimer - The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Food and Drug Administration.

Inhalation of harmful and potentially harmful constituents (HPHCs) from electronic nicotine delivery systems (ENDS) may lead to various adverse health outcomes. An important step in a health risk assessment of inhaled HPHCs is to predict the uptake in the lung. Previously, we developed deposition models for inhalation of droplet components from an ENDS puff. There are distinct differences in modeling the fate of inhaled vapors versus droplet components – most notably the interaction of droplets and vapor with lung tissues. Unlike droplets that deposit on the lung surfaces upon contact, inhaled vapor may be absorbed by lung surfaces completely, partially, or not at all. The amount of absorption depends on vapor physicochemical characteristics such as solubility, reactivity, and diffusivity in the lung tissue. Thus, vapor transport and uptake in lung airways is closely coupled with its transport kinetics in the tissue. We developed fully coupled transport models for vapors in the lung airways and tissue for several HPHCs found in ENDS. Model predictions showed significant uptake of HPHCs in the first few airways of the lung for highly reactive and soluble constituents such as formaldehyde and acrolein. These constituents were completely absorbed after several airway generations and did not reach the pulmonary region. Conversely, less reactive or low solubility compounds, such as acetaldehyde and benzene, were able to reach the deep lung where they may more readily enter systemic circulation. The vapor dosimetry model developed in this study may be a useful tool for health scientists and regulatory bodies trying to evaluate the potential for adverse health outcomes from use of ENDS. This study was funded by the Center for Tobacco Products at the U.S. Food and Drug Administration.

Vapor Uptake and Droplet Deposition of Tobacco Product Constituents in the Upper Respiratory Tract Using Computational Fluid Dynamics

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Disclaimer - The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Food and Drug Administration.
Tobacco product use is the number one preventable cause of death in the U.S. Combustible cigarettes and electronic nicotine delivery systems contain many harmful and potentially harmful chemicals. Hence, knowledge of uptake of these chemicals in the respiratory tract can aid in predicting human health risks associated with tobacco product use. We developed steady-state, inspiratory airflow computational fluid dynamics (CFD) simulations in human and rat upper respiratory tracts (URT). Physicochemical properties were incorporated into CFD simulations to obtain either (1) vapor uptake of high vapor pressure chemicals or (2) droplet deposition of low and medium vapor pressure chemicals. CFD results of vapor uptake agreed well with in vivo experimental data, with 80% of predictions within 1 standard deviation of experimental data. For a given exposure concentration, vapor uptake fraction decreased as flow rate increased. Four chemicals exhibited vapor uptake fractions that decreased as exposure concentration increased with a given flow rate. From the CFD results, we developed semi-empirical curves to predict (1) URT vapor uptake from exposure concentration and flow rate and (2) URT droplet deposition and fraction of droplets exiting the URT, based on saturation vapor pressure, initial droplet diameter, and flow rate. The semi-empirical curves exhibited excellent fits to CFD data, with coefficients of determination from 0.9–1.0. The results of this study contribute to a better understanding of the absorption of tobacco product chemicals in the URT and of the amount entering the lower respiratory tract, which is expected to assist in predicting health effects due to tobacco product use. This study was funded by the Center for Tobacco Products at the U.S. Food and Drug Administration.

The need for dosimetry to model particle-cell interactions following their nose-to-brain translocation

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In recent years there have been novel groundbreaking physiological and toxicological discoveries involving the disposition, biokinetics and bioprocessing of inhaled ambient particles in the central nervous system (CNS) following their translocation from deposition in the upper respiratory tract of humans and laboratory rodents. These findings are consistent with the hypothesis that certain air pollution particles, depending on their physiochemical properties, are causally associated with neurodegenerative diseases. The ultrafine fraction (UFP; <100nm) have the highest deposition efficiency in the nasal airways (up to 90% for UFP < 5nm) and highest reactivity per unit surface area, which implies there may also be a greater prospect of particle-cell-interactions. From extra cerebral nasal mucosa, UFP have been shown to undergo axonal transport, reaching the olfactory bulb (OB) at the frontal base of the brain. No quantification on inhaled particle deposition rates on human olfactory mucosa have been reported; however, there is a carefully designed modeling study, estimating very low 1% deposition of the inhaled amount for 1– 2nm particles on human olfactory mucosa, and only 0.01 % for 100nm particles (Garcia et al, 2015). Establishing exposure–dose–response relationships regarding neurotoxicity of inhaled airborne pollution particles on the CNS involves determining size and deposition rate in the upper respiratory tract and quantifying translocation to the CNS. Our novel findings on human autopsied OBs using high resolution analytical imaging applications reveal copious inhaled exogenous UFP including metal, metal oxide and carbon particles in OB tissues from cohort subjects with documented neurodegeneration, from subjects exposed to battlefield-related hazardous aerosols, and also from several rodent OBs involving controlled inhalation studies that use electric spark generated ultrafine metal particles. The particle numbers, nature of particles including size, morphologies and chemistries, and locations in human OBs differ greatly, in part, due to a lifetime of exposure and of course due to different environmental exposure conditions. Most translocated UFP are < 20 nm with many particles below 3 nm. They can be found intracellularly and extracellularly, and we often see them sequestered by and hosted inside Corpora Amylacea which have been suggested previously to play a role in brain clearance mechanisms. These exogenous particles are often metal oxides although we observe phosphates and mixed metals with distinct carbon coatings. Many axons, myelin sheets, astrocytes, mitochondria and epithelial linings around blood vessels are damaged/altered near UFP, suggesting particle-cell interactions. These are also regions where abundant endogenous iron nanoparticles form and colocalize with translocated exogenous UFP in the OB. We recently discovered this also occurs in deeper brain regions including Amygdala and Cerebellum. To investigate underlying mechanisms of association between neurodegeneration and pollution we aim to gain a deeper understanding of dosimetric UFP pathways for airborne metals to the CNS.

Placental-fetal distribution of carbon particles in a pregnant rabbit model after repeated exposure to diluted diesel engine exhaust

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Background: Airborne pollution particles have been shown to translocate from the mother's lung to the fetal circulation, but their distribution and internal placental-fetal tissue load remain poorly explored. Here, we investigated the placental-fetal load and distribution of diesel engine exhaust particles during gestation under controlled exposure conditions using a pregnant rabbit model. Pregnant dams were exposed by nose-only inhalation to either clean air (controls) or diluted and filtered diesel engine exhaust (1 mg/m3) for 2 h/day, 5 days/week, from gestational day (GD) 3 to GD27. At GD28, placental and fetal tissues (i.e., heart, kidney, liver, lung and gonads) were collected for biometry and to study the presence of carbon particles (CPs) using white light generation by carbonaceous particles under femtosecond pulsed laser illumination.

Results: CPs were detected in the placenta, fetal heart, kidney, liver, lung and gonads in significantly higher amounts in exposed rabbits compared with controls. Through multiple factor analysis, we were able to discriminate the diesel engine exposed pregnant rabbits from the control group taking all variables related to fetoplacental biometry and CP load into consideration. Our findings did not reveal a sex effect, yet a potential interaction effect might be present between exposure and fetal sex.

Conclusions: The results confirmed the translocation of maternally inhaled CPs from diesel engine exhaust to the placenta which could be detected in fetal organs during late-stage pregnancy. The exposed can be clearly discriminated from the control group with respect to fetoplacental biometry and CP load. The differential particle load in the fetal organs may contribute to the effects on fetoplacental biometry and to the malprogramming of the fetal phenotype with long-term effects later in life.

Withdrawn

TITLE
Exploring the Effects of Aerosol Exposure: Dose-Response Dynamics and Potential Outcomes

AUTHORS
Taylor Jefferis, Dinny Stevens, Yanira Baldovinos, Christie M. Sayes

The air we breathe is contaminated with mixtures of potentially hazardous substances that can result in acute or chronic health effects depending on the inhaled dose. Previous models have tested the effects of aerosolized substances on *in vitro* lung cell models using a known potential dose. This work expands upon earlier studies by developing laboratory experimental methods to measure four dose types: (1) potential dose, (2) applied dose, (3) internal dose, and (4) biologically effective dose. A co-culture human lung model using A549 epithelial cells and THP-1 immune cells was developed for aerosol exposure testing of occupational-relevant methacrylic acid (MAA). To control for the initial dose, a dry particle generator and nebulizer were utilized for aerosol production of each chemical and mixture. The produced aerosol was characterized by collecting particle size distribution data using the Scanning Mobility Particle Sizer (SMPS) and Optical Particle Sizer (OPS). Post-exposure, the applied and internal dose were measured using mass spectrometry to analyze samples of spent cell culture media and cell lysate. Future work should include method development involving inhalation dosimetry models to measure biologically effective doses. This work will aid in bringing together the efforts of hazard identification and dosimetry research to develop a comprehensive risk assessment of aerosol toxicants.

TITLE
Lung surfactant inhibition and cytotoxicity over increasing dry particle aerosol dose at the air-liquid interface

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Industrial processes generate chemicals that have the potential to be aerosolized and inhaled by workers, thereby posing health risks. Traditional toxicology methods employing animal models cannot keep up with the pace of emerging hazards. Nascent *in vitro* practices face challenges regarding translatability to the real world. To address this critical gap, this study demonstrated a workflow utilizing aerosol characterization in a more realistic exposure scenario: dry powder aerosolization onto the air-liquid interface of lung cells. This study delves into biophysical aspects of lung function by examining lung surfactant inhibition. A set of particulates was selected for investigation, including aluminum, aluminum oxide, carbon nanotubes, diesel particulate matter, and colloidal silica. Particles were respirable, with mean aerodynamic diameters ranging from 111 to 162 nm by number and 369 to 2884 nm by mass. Carbon nanotubes and colloidal silica were identified as surfactant inhibitors. Aerosol doses reduced cell viability up to 38%, with the most pronounced effects observed in response to exposure to aluminum and diesel particulate matter. Dry particle exposure at the air-liquid interface shows promise even at low doses, compared with nebulization or inoculation to submerged cultures. Our findings underscore the potential of this innovative approach for assessing the hazards of aerosolized particulates and emerging contaminants, offering a more accurate representation of real-world exposure scenarios.

Applying two alternative exposure methods for toxicity screening of e-liquid binary mixtures

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Vaping consists of using electronic cigarettes (or e-cigs) to heat complex mixture solutions known as e-liquids into inhalable aerosols. In 2019, the CDC defined a new public health crisis known as EVALI due to increased hospitalizations of patients. As a result, investigations into the potential toxicity associated with vaping products have increased. We aimed to investigate and utilize two alternative exposure methods to assess e-liquid toxicity in human lung *in vitro* models. The first method tested two unheated e-liquid ingredients on two human lung epithelial cell models. After exposure to several concentrations of the mixtures, cytotoxicity, and chemical interactions were assessed. Cytotoxicity was measured using LC_{50} dose-response curves of both single constituent and binary mixture solutions. The 80:20 % v/v mixture (diluent: flavoring agent) resulted in higher toxicity than the 97:3 % v/v mixture. Isobolographic analysis was used to assess the chemical interactions of binary mixtures. Each mixture resulted in an antagonistic interaction. The second method utilized a low-cost condensate collection system for condensate sample collection after vaping a binary e-liquid mixture. Chemical analysis of the condensate sample for byproduct identification was conducted using Nuclear Magnetic Resonance (NMR) spectroscopy. Diluted condensate samples were tested for induction of cytotoxicity. Toxicity was assessed by measuring (1) viability and (2) oxidative stress. Viability was measured via the MTS assay, and oxidative stress was measured via the ROS-Glo H2O2 assay, which measures hydrogen peroxide levels. In condensate-treated cells, decreased viability and production of hydrogen peroxide resulted as compared to a control of cells in media. Testing of vaping mixtures before and after heating is needed to provide information regarding their impact on human health. Although much debate surrounds the exposure techniques employed for studying vaping products, our work offers two techniques to assess different ratios and concentrations of vaping solutions.

AeroSolvedSystem: A Novel Approach for Aerosol Dosimetry Calculations

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Keywords: dosimetry, aerosol physics, reduced-order modeling
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Predicting inhalation aerosol dosimetry is challenging due to the complex and interlinked nature of aerosol physics processes taking place within the intricate and temporally changing geometry of the airways [1]. Two main approaches are used for the computational prediction of aerosol inhalation doses: Computational Fluid Dynamics (CFD) and whole lung dosimetry modeling (e.g., MPPD, ICRP) [2-4]. While CFD approaches provide a detailed description of the aerosol physics processes, including specific regions of the airways (e.g., AeroSolved [5] developed for liquid evolving aerosols), they are computationally intensive and do not allow a comprehensive representation of the lung's geometry. In contrast, the available whole lung models, although computationally very efficient, lack detailed aerosol dynamics representation and take advantage of existing empirical correlations.

Here we introduce AeroSolvedSystem, a novel approach to simulate aerosol dynamic flows in a reduced-order system- code framework. This approach substitutes the detailed 3D geometrical description with a 0D (or 1D) description of each component/element of the system. The platform is for general purpose given the complex aerosol physics of CFD AeroSolved and expands capabilities of available whole lung models implementations (Figure 1a). As such, AeroSolvedSystem enables a modular block-based platform implementation that can simulate complex aerosol dynamics in whole systems like human lungs (Figure 1b).

The newly developed capabilities allow fast simulations of evolving multispecies aerosol mixtures (Figure 1c). Apart from delivering detailed regional lung aerosol deposition databases on the local particle size distribution, the model calculates the mass transfer between the gas and liquid (particulate) phases. We present simulations that result from the aerosol particles' evolution in the human lungs when subjected to different inhalation protocols, temperature, and humidity influence, including gas-liquid phases partitioning, followed by extracted aerosol dosimetry data in distinct lung regions and compare to available literature data.

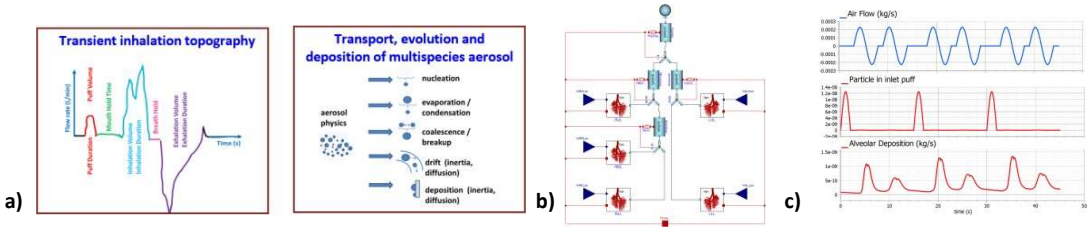

Figure 1. Graphical user interphase of a 5-lobe lung modeled with the block-based capabilities of AeroSolvedSystem a) with newly developed capabilities applicable to the whole lung modeling approach b). A 50-second (s) snapshot of results showing imposed inhalation pattern (top) with aerosol injection frequency (middle) and resulting time-captured alveolar deposition mass flux (bottom) c).

We acknowledge the software modelling support from Andrea Bartolini (Dynamica) within the Modelica framework as the computational engine for the AeroSolvedSystem platform.

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[1] Tsuda A., Henry F.S., Butler J.P. Particle transport and deposition: basic physics of particle kinetics. (2013), *Compr Physiol,* **3(4)**:1437-71.
[2] ICRP. Human respiratory tract model for radiological protection. (1994), *Ann ICRP,* **24 (1-3)**:1-482.
[3] Anjilvel, S., Asgharian B. A multiple-path model of particle deposition in the rat lung. (1995), *Fundam Appl Toxicol,* **28(1)**:41-50.
[4] Kolli, A.R., Kuczaj, A.K., Martin, F., Hayes, A.W., Peitsch, M.C., & Hoeng, J. Bridging inhaled aerosol dosimetry to physiologically based pharmacokinetic modeling for toxicological assessment: nicotine delivery systems and beyond. (2019), *Crit Rev Toxicol,* **49(9)**:725-741.
[5] Lucci F., Frederix E., Kuczaj A.K. AeroSolved: Computational fluid dynamics modeling of multispecies aerosol flows with sectional and moment methods. (2022), *J Aerosol Sci*, **159**:105854.

Evolving Aerosol Dosimetry Integrated Physiologically Based Pharmacokinetic Modeling for Inhalation Delivery Systems

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The respiratory tract is geometrically heterogeneous with varied branching patterns, airway tissue thickness, blood perfusion, cellular composition, and physiologically-dictated airway lumen flowrate conditions. While an aerosol entering the airway lumen undergoes continuous evolution resulting in differential regional deposition patterns, the complex interplay of varied pulmonary kinetic processes such as regional absorption, clearances, and retention kinetics influence the fate of its chemical constituents. Integrating aerosol dosimetry to physiologically-based pharmacokinetic (PBPK) models by including various kinetic processes enables a more realistic prediction of local and systemic pharmacokinetics (PK) of inhaled chemicals [1, 2].

We developed AeroSolvedSystem, a novel computational platform to simulate flow of inhaled aerosol by including aerosol transport, evolution, and deposition in a reduced-order system-code framework and combined it with a state of the art ion-trapping based PBPK model for nicotine [3]. This approach allows the integration of a newly developed deterministic whole-lung model comprising a comprehensive implementation of thermodynamically dependent evolution of multispecies mixtures of aqueous aerosols providing a more reliable prediction of aerosol evolution in the airway lumen. The new tool offers unique capabilities since it considers the transient and complete human inhalation topography profiles and estimates the regional aerosol deposition resulting both from evolving particle multiphase dynamics and resulting gas phase absorption (Figure 1). The integrated PBPK model uses real-time regional deposition and absorption kinetics of nicotine to predict local and systemic PK.

The newly developed computational capabilities satisfactorily predict existing clinical nicotine PK resulting from various inhalation-based delivery systems [4-6]. The predictions of multiphase aerosol evolution inside the human respiratory tract will be discussed in relation to the resulting PK profiles. Introducing these developments will bridge inhaled evolving aerosol dosimetry with PBPK modeling, which enables a more reliable pharmacological and toxicological assessment of exposure-responses.

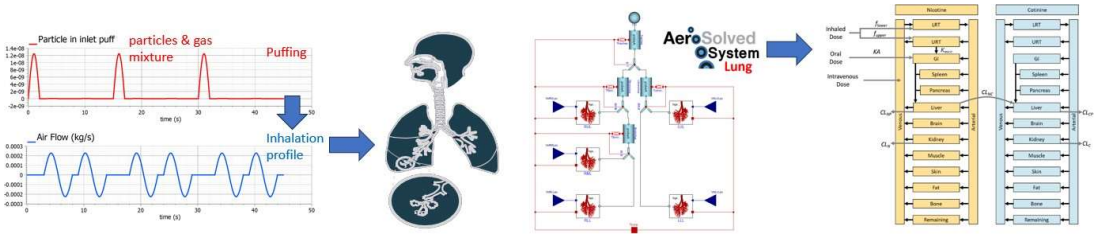

Figure 1. Graphical representation of the developed capabilities combining human topography aerosol inhalation with the whole lung modeling approach, and multi-compartmental physiologically based pharmacokinetic model.

Philip Morris International is the sole source of funding and sponsor of this research.

[1] Schroeter J., Asgharian B., Price O., Parks A., Oldson D., Fallica J., Erives G., Li C., Rass O., Harvanko A., Peters K., Chemerynski. S. Simulation of electronic nicotine delivery systems (ENDS) aerosol dosimetry and nicotine pharmacokinetics. (2024) *Comp Toxicol*, **3**:100322.

[2] Kolli, A.R., Kuczaj, A.K., Martin, F., Hayes, A.W., Peitsch, M.C., Hoeng, J. Bridging inhaled aerosol dosimetry to physiologically based pharmacokinetic modeling for toxicological assessment: nicotine delivery systems and beyond. (2019), *Crit Rev Toxicol,* **49(9)**:725-741.

[3] Kolli, A.R., Calvino-Martin, F., Kuczaj, A.K., Wong, E.T., Titz, B., Xiang, Y., Lebrun, S., Schlage, W.K., Vanscheeuwijck, P., Hoeng, J. Deconvolution of Systemic Pharmacokinetics Predicts Inhaled Aerosol Dosimetry of Nicotine. (2023), *Eur J Pharm Sci*, **180**:106321.

[4] Fearon, I.M., Eldridge, A., Gale, N., Shepperd, C.J., McEwan, M., Camacho, O.M., Nides, M., McAdam, K., Proctor, C.J. E-cigarette Nicotine Delivery: Data and Learnings from Pharmacokinetic Studies. (2017), *Am J Health Behav*, **41(1)**:16-32.

[5] Gourlay, S.G. and Benowitz, N.L. Arteriovenous differences in plasma concentration of nicotine and catecholamines and related cardiovascular effects after smoking, nicotine nasal spray, and intravenous nicotine. (1997), *Clin Pharmacol & Ther*, **62(4)**:453-63.

[6] Rose, J.E., Behm, F.M., Westman, E.C., Coleman, R.E. Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: implications for addiction. (1999), *Drug Alcohol Depend*, **56(2)**:99-107.
implications for addiction. (1999), *Drug Alcohol Depend*, **56(2)**:99-107.

Deposition of Evolving Multispecies Aerosol Mixtures in the Airways During Transient Inhalation Including Aerosol Uptake and Mouth-hold

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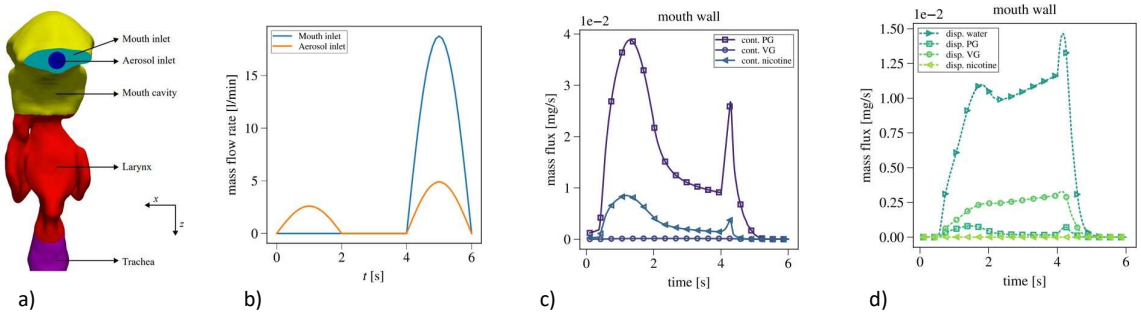
[3] Applied Mathematics, EEMCS, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands Keywords: upper respiratory airways, aerosol delivery, aerosol physics, computational fluid dynamics Presenting author email: arkadiusz.kuczaj@pmi.com

Computational Fluid Dynamics simulations of aerosol deposition in the human airways have been conducted for many years and developed for a variety of applications [1,2]. The increasing complexity of aerosol delivery (i.e., evolving multiphase aerosol physics with particles consisting of many substances with distinct volatility potential, and in the presence of temperature and humidity gradients) requires the development of complex fluid mechanics codes. We tackle this challenge by continuously improving the capabilities of AeroSolved [3], a publicly available code for simulating aerosol transport, evolution, and deposition. With the newly developed boundary conditions that allow simulations of evolving liquid particles in the presence of thermodynamically coupled wet surfaces, we evaluate the aerosol evolution and deposition at realistic transient inhalation profiles in a human intrathoracic airway geometry (Figure 1a). Aerosol mixture and inhalation conditions are typical of e-vapor consumer products. First, 50 mL of aerosol mixture, including equilibrated gas and liquid phases at 50 °C, is injected for 2 seconds through a circular inlet into the mouth cavity. After a mouth hold of 2 seconds, 450 mL of humidified air at 25 °C is inhaled for 2 seconds, allowing the aerosol to mix and evolve (Figure 1b). At the airway surface, the temperature is fixed at 37 °C and water relative humidity is varied up to 98%. Under the tested conditions, the local thermodynamic state at the airway surface triggers significant aerosol dynamics resulting in water evaporating into the domain and particle condensation in the vicinity of the boundary layer with consequent particle growth and equilibration of the partitioning of the main aerosol substances between the phases (Figure 1c-d). The improved capabilities allows the assessment of condensational growth and subsequent deposition mass fluxes resulting from impaction, sedimentation, and diffusion for liquid-evolving particles and diffusion- driven deposition of substances present in the gas phase.



a) b) c) d)

Figure 1. Visualization of the airway domain (Zhang *et al.* 2012) with subdivided regions (a), imposed temporal inhalation profile (b), and corresponding deposition rate of aerosol mixture substances present in the gas (c) and liquid (d) phases. Aerosol consisted of water, vegetable glycerol (VG), propylene glycol (PG), and nicotine.

Philip Morris International is the sole source of funding and sponsor of this research.

[1] Longest PW., Holbrook LT. In silico models of aerosol delivery to the respiratory tract - development and applications. (2012), *Adv Drug Deliv Rev*, **64(4)**:296-311.

[2] Rostami AA. Computational modeling of aerosol deposition in respiratory tract: a review. (2009), *Inhalation Tox,* **21(4)**:262-290.

[3] Lucci F., Frederix E., Kuczaj A.K. AeroSolved: Computational fluid dynamics modeling of multispecies aerosol flows with sectional and moment methods. (2022), *J Aerosol Sci*, **159**:105854.

[4] Zhang Z., Kleinstreuer C., and Hyun S. Size-change and deposition of conventional and composite cigarette smoke particles during inhalation in a subject-specific airway model. (2012) *J Aerosol Sci*, **46**:34-52.

InHALES: A Mechanical Replica of the Human Respiratory Tract for Advanced *In Vitro* Inhalation Studies

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Keywords: in vitro, inhalation toxicology, aerosol exposure system
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An challenge in aerosol inhalation toxicity or efficacy assessments is appropriate and accurate determination of the delivered dose. *In vitro* exposure is pivotal in understanding aerosol deposition in the human lung and its subsequent biological responses. Existing *in vitro* exposure systems fail to replicate the aerosol inhalation processes that naturally occur inside the airways. Consequently, aerosol exposure of cell tissue cultures takes place under different conditions than in a respiratory tract [1]. To overcome this limitation, we have engineered InHALES (Figure 1A), a mechanical analogue of the human respiratory tract that delivers aerosols under more realistic conditions. In addition, by emulating the anatomical intricacies of the respiratory system, we aim for the aerosol delivery and deposition to be analogous to that inside the human lung, resulting in representative quantification of delivered doses.

InHALES comprises three models (Figure 1B): oral cavity (1) actively generates aerosols from medical or consumer inhalation products, conducting airways (2) linking the oral cavity and lung lobes, housing cell cultures, sensors, and probes, and lung lobes (3) simulating inhalation and exhalation processes and accommodating up to 5.5 L of air volume. The airway model adheres to Weibel's morphometry [2], replicating the conducting airways down to the 10^{th} generation bifurcation. The lung lobe model integrates ceramic elements to mimic the finer branching and surfaces of alveolar sacs. The entire system is housed within a climatic chamber, maintaining a consistent temperature of 37 °C.

The prototyped system, described in Steiner *et al.* [3], supports programming of any physiological human inhalation patterns and ensures reliable aerosol delivery to cell cultures. Current studies are underway to evaluate its capacity to mimic key characteristics of aerosol deposition in the lungs, including retention, particle size-specific deposition, and gas-to-particle ratios. This work represents a significant advancement in the field of *in vitro* inhalation sciences.

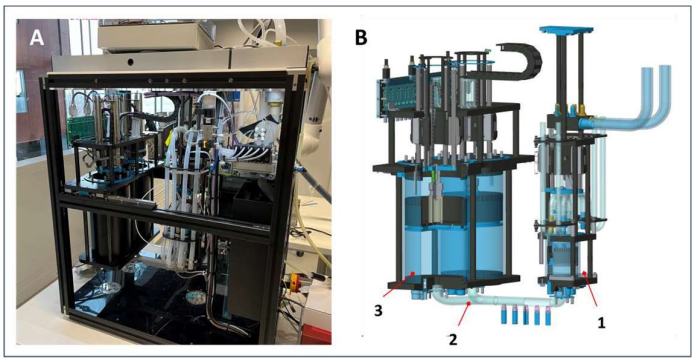

Figure 1. The InHALES system prototype (version 1.0) (A) with a 3D CAD section (B) showing models of the oral cavity (1), conducting airways (2), and the lung lobes (3). The ceramic elements within the lung lobes are not shown. Philip Morris International is the sole source of funding and sponsor of this research.

[1] Secondo, L. E., Liu, N. J., Lewinski, N. A. Methodological considerations when conducting in vitro, air–liquid interface exposures to engineerednanoparticle aerosols. (2017). *Crit Rev Tox*, **47(3)**:225–26.

[2] Weibel, E.R. Morphometry of the Human Lung. (1963). Springer Berlin, Heidelberg.

Steiner, S., Herve, P., Pak, C., Majeed, S., Sandoz, A., Kuczaj, A., Hoeng, J. Development and testing of a new-generation aerosol exposure system: The independent holistic air-liquid exposure system (InHALES). (2020). *Tox in vitro* **67**:104909

Linking Dosimetry, Pharmacokinetics, and Long-Term Health Implications in Mice Exposed to Cigarette Smoke and Pod-Mod E-cigarette Aerosols

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Electronic cigarette (EC) usage has become popular amongst naïve users, yet the long-term health implications remain unknown. To investigate dosimetry and impact on health, we performed experiments with 3R4F research cigarettes (CIG, University of Kentucky) or tobacco-flavored JUUL™, 3% nicotine. Particle sizes and concentration were measured with EEPS (TSI) and a MicroDust Pro (Casella), respectively. Mice respiration waveforms were collected with a plethysmography device. Particle dosimetry was calculated with a modified version of the trumpet model, uniquely accounting for breath holds experienced by exposed mice. Apoe$^{-/-}$ mice were exposed to CIG smoke (28, 56, 70, or 84 mins) or EC aerosols (15, 30, 60, 120 mins), and blood was collected for cotinine analysis. Elimination was studied 30, 90, and 150 mins after exposure. Pharmacokinetic analysis was performed to determine cotinine survival functions. Following, mice were chronically exposed to air, CIG smoke, or EC aerosols. Airway resistance (R_N), elastance (H), and tissue resistance (G) were determined (flexivent, Scireq). Count median diameters were 178 (CIG) and 116 (EC) nm and mass concentration was lower in EC aerosols (0.31 mg/L) when compared to CIG smoke (0.55 mg/L). Maximum concentration was larger in CIG (6.23, [3.90, 8.73] 1/mL) compared to EC (4.57, [3.14, 7.35] 1/mL) exposed mice, and cotinine absorbed and eliminated faster in EC exposed mice. As CIG-exposed mice augment their breathing, 13 times more mass deposited in mice exposed to EC aerosols. R_N, G, and H were elevated in 24-week CIG and 16-week EC-exposed mice. No change was noted in EC mice at 24 weeks. Parenchyma tissue was thicker in both exposure groups at 24 weeks. This study highlights that while more particles deposit in EC-exposed mice, remodeling of the respiratory system is more severe in CIG-exposed mice. Nonetheless, this study highlights the need to further study the health implications of ECs.

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Smoke Dosimetry Computed with a Hybrid Lung Model in Mice Exposed to Wildland Fire Smoke

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Wildland fire smoke poses a significant health problem as it contributes to about a third of air pollution. Mouse models enable for the long-term health consequences to be studied, where the biological underpinnings of fire smoke exposure can be elucidated. We recently developed a mouse exposure model to investigate the health effects of repeated exposure to wildland fire smoke, designed to model a mid-length wildland firefighter career[1]. To better understand dosimetry in the mouse model, here we perform computational particle deposition calculations by utilization of a hybrid 3D-1D model. Airways are created (Simvascular) from μCT images collected at the end of expiration. The 3D model is connected to a 1D model to compute airflow and transport in the airways not resolved through μCT images. Experimentally measured data of mouse lung mechanics, mouse breathing patterns, and smoke particle size distribution are used to parameterize the computational models. We found that the augmented breathing pattern adopted by mice during wildland fire smoke exposure led to an approximately 25% increase in the total deposition fraction of inhaled particles. Nonetheless, the significantly lower minute volume during smoke exposure ultimately decreased total deposited mass compared to a normal breathing pattern, with most of the particles depositing in the acinar region of the lung. Furthermore, most particles that deposited within the 3D model did so in the nasal cavity, with notable deposition occurring in the olfactory region. This computational investigation improves our understanding of the regional deposition of wildland fire smoke particulates in the mouse respiratory tract, which is important for understanding regional changes in respiratory health because of wildland fire smoke inhalation and identifying how the particles access other organ systems.

Reference: 1: Eden et al. *Science of the Total Environment.* 2023: 861: 160609

Funding: DHS/FEMA: EMW-2017-FP-00446 and NIH/NIEHS: R01E5033792

Inhaled Aerosol Dosimetry in the Context of a Source-Exposure-Dose-Health Effects Paradigm

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Over the past century substantial progress has been made in understanding how airborne chemicals, physical agents, radioactive materials and microbes in the workplace or ambient environment, or from consumer use, can cause adverse health effects over and above those occurring spontaneously, or from other causes. As the science has advanced, a unifying paradigm has emerged linking "Sources-Exposure-Dose-Health Responses". This paradigm provides a basis for planning and conduct of research, interpretation of scientific findings, integrating and synthesizing the science for conducting risk/safety analyses and for developing policy, regulations, standards, and practices that will limit adverse health outcomes. This conference on "Inhaled Aerosol Dosimetry" focuses on the critical link between Exposure and Dose in the paradigm. Past progress in understanding the adverse health outcomes of exposure to airborne agents as well as their application in the diagnosis and treatment of disease has come from observational studies of human populations and planned experimental studies using humans, laboratory animals, and cellular and sub-cellular systems. Application of these findings to diverse human populations requires extrapolations among species and across Exposure/Dose levels that are aided by mechanistic understandings of the linkages and the extrapolations. The current emphasis on Replacement, Reduction and Refinement of animal usage requires that new approaches be developed for using data from cellular and sub-cellular systems in the paradigm described above. This will inevitably require new computational modeling approaches. Conference attendees need to consider the information presented at this Conference within this paradigm.

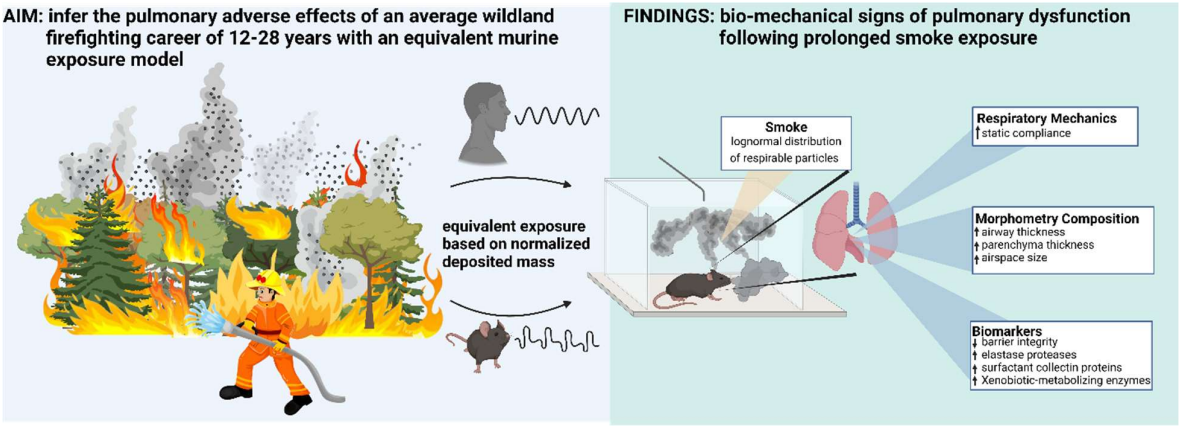
Modeling and evaluating the pulmonary impacts of a wildland firefighting career through equivalent murine exposure

C. Yazbeck[1], M. Eden[1], J. Matz[1], M. Gollner[2], C. Bellini[1], J. M. Oakes[1]; [1]*Bioengineering, Northeastern University, Boston, MA, USA*, [2] *Mechanical Engineering, University of California, Berkeley, CA, USA.*

Rationale: Wildland firefighters (WLFFs) are increasingly subjected to the unavoidable risks of wildfire smoke exposure as the frequency and severity of wildfires continue to rise. Concerns about occupational health hazards are significantly heightened, particularly as wildfire smoke particulates (PMs) are linked to a poorly understood decline in pulmonary function. In our study, we aim to investigate the underlying biomechanical impacts of an average 12-28 years WLFF career through an equivalent murine model. **Methods:** We utilized a dosimetry model to determine an experimental protocol that matches the cumulative PM dose deposited in the lungs of WLLFs and apolipoprotein E-deficient (ApoE-/-) mice. The equivalency of 3 g of deposited PM mass in WLLFs after 7,200 work hours and 6 g after 14,400 work hours was established by exposing ApoE-/- mice to smoldering Douglas fir smoke (DFS) at an approximate PM concentration of 40 mg/m³ for 2 hours/day, 5 days/week, for 8 weeks, and 16 weeks, respectively. Following the prolonged exposure, the mice were intubated endotracheally and placed on a mechanical ventilator (flexiVent, SCIREQ) to measure pressure-volume (P-V) curves. We assessed changes in tissue morphology with histological staining, and pulmonary biomarkers with immunofluorescence. **Results:** PMs in smoldering DFS were found to follow a unimodal lognormal distribution, with a count median diameter of 110 ± 20 nm and a geometric standard deviation of 1.47 ± 0.03. Consequently, after 16 weeks of DFS exposure the P-V curve shifted upward and leftward and static compliance was significantly higher compared to air controls, but hysteresis remained unchanged. This observation was supported by a significant increase in the thickness of the airway epithelium and alveolar septa, along with airspace enlargement. In addition, after 16 weeks of exposure, there was a significant decrease in the mean fluorescence intensity of the tight junction marker ZO-1 in both the parenchyma and airway epithelium, without a corresponding expression of ongoing epithelial-mesenchymal transition marker N-Cadherin. Both neutrophil elastase and surfactant protein-D markers exhibited a transient response. **Conclusion:** The decline in lung functions observed in WLLFs could be attributed to damaged airways and degraded alveolar septa in response to inflammation, leading to emphysema.

Keywords: Occupational health, wildfire smoke inhalation, particulates, lung injury

Graphical abstract:



E-Vapor Enters Paranasal Sinuses: An In Vitro Evaluation Using Patient-Specific 3D-Printed Models

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Biomedical Engineering, University of Massachusetts, Lowell, MA

Abstract:

Background: This study aims to assess the effectiveness of e-vapor entering and filling the paranasal sinus cavities, particularly the maxillary sinuses. Demonstrating this potential is important as e-vapor-based aerosols could serve as a vehicle for delivering active pharmaceutical ingredients (APIs) directly to the paranasal sinuses, improving treatment outcomes for conditions such as rhinosinusitis and nasal allergies. An effective sinus delivery addresses challenges posed by conventional media like nasal drops, sprays, and mists, which often fail to penetrate the small ostia and reach the sinuses.

Materials and Methods: In vitro experiments utilized multiple patient-specific, transparent, 3D-printed nasal cavity models, including a model representing post-uncinectomy conditions, to evaluate the effectiveness of intranasally administered e-vapor in entering and depositing aerosols within the sinuses. The e-vapor was dispensed from a custom-made vapor dispensing device utilizing a mini fog machine under unidirectional and bidirectional airflow conditions. Visualizations from various nasal model views, angles, and lighting conditions were recorded. Particle size distribution measurements of the administered e-vapor were conducted using a laser diffraction particle size analyzer. Supplementary tests compared the effectiveness of e-vapor with traditional intranasal administration techniques, including a multi-dose nasal spray pump, nasal squeeze bottle, mesh-nebulizer, and humidifier.

Results: The study demonstrates that e-vapor, composed solely of vegetable glycerin (VG) and propylene glycol (PG), effectively enters and deposits in the paranasal sinuses under specific administration parameters. E-vapor particle size measurements revealed a mean particle size ranging from 2.895 to 3.359 μm, with a median particle size (D50) averaging 2.963 μm. The speed of vapor entering the paranasal sinuses is directly proportional to the ostia size; larger ostia result in faster sinus entry. A continuous moderate flow of vapor is necessary to gradually fill the paranasal sinus cavities due to the small orifice openings. The dynamics and mechanisms of vapor entry involve maintaining a positive pressure gradient across the ostial canal. This gradient, facilitated by an appropriate flow rate, generates sufficient pressure to drive the vapor into the sinuses, where gravitational forces and recirculation currents further enhance the deposition of vapor aerosols. Comparative tests showed that traditional delivery devices exhibited limited penetration and failed to reach the sinus cavities effectively.

An in vitro fibrosis mini-lung with real-time monitoring of cell mechanics for improved clinical relevance

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Helmholtz Zentrum Munchen

ABSTRACT NOT AVAILABLE

AI-driven aerosol dosimetry and regional distribution in murine lungs

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Helmholtz Munich, Institute of Lung Health and Immunity, 85764 Neuherberg, Germany

ABSTRACT NOT AVAILABLE

Aerosol Science Development Fostered by the Manhattan Project, Atomic Energy Commission and Department of Energy Programs

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In 1940 Seaborg used the Cyclotron to bombard Uranium (U) with deutrons producing a new fissionable element—Plutonium (Pu) with a half-life of 24,390 years. The Manhattan Project (MP) was initiated in 1942 to develop atomic bombs fueled by Pu–239 and U-235. Concern arose for related health hazards and research began in laboratories at the Universities of California-Berkeley, Chicago, Rochester, and at new laboratories at Los Alamos, NM, Hanford, WA and Oak Ridge, TN. A new era of aerosol science began. The first atomic bomb was detonated in NM in July 1945. In August 1945 atomic bombs were detonated over Hiroshima and Nagasaki, Japan ending WWII. In 1947 the MP was transferred to the US Atomic Energy Commission (AEC) and research on health effects of radiation expanded. Following WWII, Atomic Bombs were tested in the Pacific and in Nevada. Russia developed and tested Atomic Bombs with other countries following. Concern over airborne radioactivity increased. The University of Utah in 1950 was funded by the AEC to conduct lifespan studies on Pu–239 and other radionuclides. In the late 1950s, development of nuclear reactors for electricity increased concern for airborne radionuclides. In 1960 a new laboratory in NM operated by the Lovelace Medical Organization began focusing on inhaled fission product radionuclides. In 1971 the AEC became the Energy Research and Development Administration (ERDA), and the Nuclear Regulatory Commission (NRC). In 1977 ERDA became the Department of Energy (DOE). In the 1970s concern over use of Pu–239 in reactors and Pu–238 in Space Nuclear Auxiliary Power devices lead DOE to initiate lifespan studies with Pu–238 and Pu–239 at Lovelace. In the 1980s these laboratories shifted attention to health effects associated with use of fossil fuels. The contributions of these laboratories to aerosol science has been immense!

Title: **Methods for Collecting and Evaluating Morphology and Quantity of Inhalable Airborne Microplastic Particulates**

David Chalupa[1], Samantha Romanick[2], James McGrath[2], Alison Elder[1]
Departments of [1]Environmental Medicine, and [2]Biomedical Engineering, University of Rochester; Rochester, NY

There is a rapidly growing amount of literature regarding human environmental exposures to microplastic particles and fibers, specifically in water sources and consumer products. Less is known regarding inhaled microplastics. Studies have identified microplastics in indoor and outdoor air samples, but whether these particles are of a size that can deposit in the respiratory tract is only starting to be explored. Therefore, we are currently developing methods to readily identify inhalable microplastic particles and fibers, focusing specifically on PM_4 and smaller, in indoor and outdoor air.

We used optically clear silicon nitride nanomembranes (Simpore Inc., Rochester, NY) to collect air samples with the intention of morphology and count analyses. These nanomembranes allow for short term sample collection on an optically transparent non-plastic medium for microscopic examination. Nile red stained plastics were separately identified from other particles such as cellulosic material (Typan blue stain) and proteinaceous particles using an epifluorescence microscope. We have also used traditional methods of concurrent air sampling for additional characterization of inhalable particles. This includes size selective inlets (PM_4 or $PM_{2.5}$) and cascade impactors. The bi-modal mass median aerodynamic diameter (MMAD) for air collected in some indoor environments included a home laundry (0.9 and 6.6 microns, 16.5 ug/m^3), and a machine shop (1.5 and 8.9 microns, 26.0 ug/m^3). Simpore filters were also used to collect and stain particles to indicate the amount by count or ratio of plastic stained particles to other particles collected. Future directions include testing more outdoor air locations spatially or regionally across the Rochester area and temporally over seasons.

Influence of Aerosol Deposition, Molecular Weight and Nonclinical Studies on Inhaled Biologics Development

Ron Wolff

RK Wolff Safety Consulting Inc.

For systemic therapies, inhaled biologics are influenced by their aerosol deposition site and molecular weight. Since they have low oral bioavailability use of aerosol devices that enhance deep lung deposition are optimal. Molecular weight also influences uptake with generally lower absorption into

blood as molecular weight increases. Nonclinical studies will be reviewed in support of development of inhaled biologics including proteins, oligonucleotides, vaccines, and antibodies. There will be discussion of the main types of effects seen in the lungs with these agents and some of the general and specific features of their effects in lungs. The challenge of conducting studies in animals with human molecules will be outlined. Influence of excipients and formulation will also be discussed. This review is intended to provide perspective for current and future development of inhaled biologics.

Generation and Characterization of Libby Asbestos Aerosol During Chronic Inhalation Toxicology Studies

Matthew D. Stout[1], Georgia Roberts[1], Pei-Li Yao[1], Amit Gupta[2], Jamie S. Richey[2], John R. Shaw[2], Dawn Fallacara[2], Anbo Wang[3], Alex Easley[3], Karen E. Elsass[3], Benjamin J. Ellis[4], Robyn L. Ray[5]

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Inhalation exposure to asbestos has been shown to cause lung cancer, mesothelioma, and non-neoplastic lung and pleural disease in humans. The Division of Translational Toxicology at the National Institute of Environmental Health Sciences is conducting toxicology studies to better understand the range of health effects, and air concentrations that result in health effects, across fiber types, with Libby Amphibole asbestos (LAA) selected as the initial fiber sample to be tested as part of this effort. Human exposure to LAA occurs as a result of vermiculite mining in Libby, MT. Notably, LAA was collected and processed to be representative of human exposure in Libby. Based on short-term study findings, a chronic study was designed to evaluate the toxicity on LAA in rats exposed to concentrations of 0, 0.1, 0.3, 1 and 3 mg/m^3 by nose-only inhalation exposure for up to 3 hrs/day, 5 days/week for 2 years. Two additional groups of male rats were exposed, such that the cumulative concentration x time (C x T) matched that of the 0.3 or 1.0 mg/m^3 group. LAA was aerosolized using a single slide bar aerosol generator, which metered the desired quantity of test material to generate aerosol at controllable concentrations. The resulting aerosol was conveyed to the five exposure carousels by high-velocity transportation line and air ejector pumps. The spatial uniformity of aerosol concentration within each exposure carousel was within ≤ 5%. Mean daily exposure concentrations were within 3% of the target concentration. The overall mean relative standard deviation (RSD) of daily means was ≤ 8% for all groups. The mean nose port temperature, relative humidity, and the carousel inlet flows were within the respective predefined range. Samples were collected from the bulk LAA test material and at the nose ports of each exposure carousel, and analyzed to confirm fiber identity and determine fiber size distribution. These analyses indicated that the aerosolized material was not altered during generation and transport through the exposure system. Overall, stable LAA concentrations that were chemically and physically similar across exposure groups and representative of human expsoures were delivered to the study animals.

Characterization of an Air Liquid Interface (ALI) Exposure System to Support New Approach Methodologies for Inhalation Toxicology Assessments Using the Flavoring Agent 2,3-Pentanedione

Amit Gupta[1], Bob Moyer[1], Stephanie Pearson[1], Ray Devine[1], Jamie Richey[1], John Shaw[1], Janae Newswanger[1], Cassady Griffin[1], Dawn Fallacara[1], William Gwinn[2], Pei-Li Yao[2], and Georgia Roberts[2]
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The desire to improve the efficiency of toxicity testing and provide more accurate predictions of human health effects is accelerating a shift towards implementing new approach methodologies (NAMs) and the use of "big data" for risk assessment and human relevance. We are interested in integrating multi-omics next generation sequencing ([NGS]; i.e., transposase-accessible chromatin with sequencing [ATAC-Seq] and methylation status) with in-vitro inhalation exposure systems to investigate test article-associated changes in gene expression networks with the aim to further inform a better prediction of human health outcomes. Specifically, airway cultures derived from primary human tracheobronchial epithelial cells (3D organotypic cell culture models) grown at the air liquid interface (ALI) were tested. The artificial butter flavoring agent 2,3-pentanedione (PD) was selected as the test article for the development and characterization of an in-vitro inhalation exposure system.

A major challenge of in-vitro inhalation exposure systems is the controlled delivery and accurate characterization of the exposure atmosphere. To enable accurate and efficient measurement of chemical (vapor) concentrations at the ALI, we have integrated an online gas chromatograph (GC) with a multiport rotation autosampler valve and a commercially available exposure system (i.e., VITROCELL® 48 2.0 plus). To generate exposure atmospheres, PD was injected into a vaporization column using a syringe pump. Air, entering from the bottom of the column, vaporized the chemical and carried the vapor to the inlet of the ALI exposure system. Humidified dilution air was added at the inlet of each row of the ALI exposure manifold to dilute the atmosphere to various target concentrations. The online GC was used to monitor PD vapor concentrations and determine the stability of PD at the target concentrations (0, 40, 70, and 130 ppm). The use of the multiport rotation valve enabled efficient and simultaneous real-time monitoring of the vapor concentrations as the exposures were conducted. The results demonstrated that the vapor concentrations in each row of the exposure manifold were stable throughout the exposure period and within 2% of the target. Prior to cell exposures, the uniformity of deposited dose and the vapor deposition efficiency were determined by using liquid solvent trap samples (MatTek assay maintenance medium) in each transwell. The solvent samples were analyzed for PD using gas chromatography flame ionization detection (GC-FID) with headspace sampling. The variability of PD concentration among transwells was assessed to determine the uniformity of deposited dose. Based on analytically determined deposited PD concentration in each transwell, and PD vapor concentration as measured by the online GC, the deposition efficiency of PD in the exposure system was determined.

In summary, methods were developed to establish accurate and efficient characterization of the test atmosphere in a VITROCELL 48 2.0 plus exposure system using real-time monitoring of multiple concentrations of the test article during in-vitro ALI exposures. The uniformity of deposited dose and deposition efficiency of the test article were assessed.

Dry powder inhaler deposition in the larynx and the risk of steroid inhaler laryngitis: a computational fluid dynamics study

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Abstract

Dry Powder Inhalers (DPIs) are a mainstay in the treatment of obstructive respiratory diseases, including asthma and chronic obstructive pulmonary disease (COPD). Deposition of inhaled corticosteroids in the larynx elicits local side effects, potentially leading to steroid inhaler laryngitis. The objective of this study was to estimate the dose of DPIs that are deposited in the larynx relative to other regions of the respiratory tract using computational fluid dynamics (CFD). An anatomically accurate model of the airways (mouth to main bronchi) was constructed based on medical imaging of a healthy adult. Respiratory airflow and particle transport were simulated for constant inhalation rates of 30, 45, and 60 L/min. Two turbulence models were compared, namely the large eddy simulation (LES) and the $k-\omega$ SST turbulence models. DPIs were assumed to generate an aerosol cloud with a log-normal particle size distribution characterized by the mass median aerodynamic diameter (d_{50}) and geometric standard deviation (σ_g). We compared two commercial DPIs, namely DPI 1 had a large particle size (d_{50} = 50 µm, σ_g=2.55) and DPI 2 had a small particle size (d_{50} = 2 µm, σ_g=1.99). At an inhalation rate of 30 L/min, the laryngeal dose was 1.6-to-3.8-fold higher than the bronchial dose for DPI 1, while the laryngeal and bronchial doses were similar (units of mass per unit surface area) for DPI 2 for both turbulence models. This suggests that the risk of steroid inhaler laryngitis is greater for DPIs with larger particles. Our results also highlight the influence of the turbulence model on the regional dose predictions, with the LES model predicting higher larynx-to-bronchi relative doses than the $k-\omega$ model. In summary, our CFD simulations suggest that the risk of laryngeal side effects in DPI users can be reduced by selecting DPIs with smaller particle sizes.

Standardization of lung Microphysiological System considering continuous flow exposure

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Recent advancements in lung organ-on-a-chip technology have significantly enhanced our ability to mimic in vivo lung behavior in vitro. These sophisticated models incorporate key features of the lung microenvironment, including the air-liquid interface, mechanical stretching to simulate breathing, and co-culture of multiple cell types. Typically consisting of two microfluidic channels separated by a porous membrane, these platforms allow for precise control of fluid flow, cellular interactions, and exposure to test substances.
These advanced in vitro models play a crucial role in implementing the 3Rs principle (Replacement, Reduction, Refinement) in toxicological testing and drug development. By offering a more accurate representation of human lung physiology, they have the potential to reduce reliance on animal testing, improve predictive accuracy, and accelerate drug discovery.
Recognizing the importance of standardization, Korea has initiated the development of international standards for lung microphysiological systems (MPS) or lung-on-a-chip under ISO TC 276. This presentation aims to build consensus on the standard development procedure, addressing key aspects such as device design, cell sourcing, culture conditions, and performance metrics.
A critical component of lung-on-a-chip models is the exposure system, which must accurately simulate respiratory tract exposure to various substances. We will discuss the physical requirements of lung MPS for test material exposure, including factors such as airflow dynamics, particle size distribution, and deposition patterns.
To evaluate exposure system performance, we conducted experiments using size-standard particles in a continuous flow setup. We will present an analysis of deposition uniformity across the lung-on-a-chip model, considering spatial distribution, temporal variations.
This work contributes to developing robust, standardized lung-on-a-chip platforms that can reliably predict human lung responses to various stimuli. By addressing key challenges in exposure system design and characterization, we aim to enhance the applicability of these models in toxicological assessments and drug screening processes, ultimately advancing the field of in vitro lung research.
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Computational framework for subject-specific aerosol dosimetry and internal radiation dosimetry using CFPD and Monte-Carlo transport codes.

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Abstract:

The traditional methods for performing subject-specific aerosol dosimetry using Computational Fluid and Particle Dynamics (CFPD) involve several steps, mostly involving manual intervention and requiring expertise in different areas: image segmentation, geometry modeling, CFPD, and post-processing. This work introduces a fully automated framework to streamline subject- specific particle deposition analysis within the human respiratory tract (HRT). This approach addresses anatomical and physiological variations inherent in the HRT, which are crucial for drug delivery systems, targeted respiratory therapies, and the assessment of radioactive inhaled particles from various sources.

By integrating deep learning and CFPD simulations, we have developed a computational method capable of generating individualized particle deposition profiles from 3D reconstructions of HRT geometries based on Computed Tomography (CT) scans. This study used enhanced computer vision algorithms for HRT reconstruction from CT scans, VTK libraries for geometry pre-processing for CFPD simulation (i.e., smoothing, outlet/inlet definition), Python and Java scripts for meshing and CFPD modeling for airflow and particle simulation, for both Open- Source (OpenFOAM) and commercial (StarCCM+) CFD solvers. Moreover, Python scripts were developed to generate source files for Monte Carlo simulations for internal dose assessment from inhaled radioactive particles, particularly tailored to the Particle and Heavy Ion Transport code System (PHITS).

Validation and verification of CFPD methodologies adhered to the National Program for Applications-Oriented Research in CFD guidelines. The work was benchmarked against previous in-silico and in-vitro studies and was tested on 14 different HRT geometries under a broad range of inhalation conditions, demonstrating its versatility. The PHITS simulations effectively mapped the energy deposition from inhaled radioactive particles, showcasing its applicability in radioactive exposure scenarios. Our findings demonstrate that minor HRT variations notably influence particle deposition, observing more than 30% variance in the mass and particle deposition fraction. Regarding absorbed dose, variations between 15-98% were observed when compared to ICRP uniform deposition models.

Surface area-based toxicity ranking of aerosolized nanomaterials from epithelial lung cells cultured under air-liquid interface conditions: A meta-analysis

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Respiratory toxicity of engineered nanomaterials has been extensively assessed with *in vivo* and cell-based submerged *in vitro* models. While *in vivo* testing is costly and time consuming, *in vitro* testing in submerged exposure conditions is neither representative of the physiologic conditions in the lung nor is it well controlled for cell-delivered dose. On the other hand, there is a substantial body of *in vitro* toxicity data for air-liquid interface (ALI) cultured lung epithelial cells, which might overcome some of these limitations of submerged cell culture models. We conducted a meta-analysis searching the PubMed and OpenAlex databases to investigate the effects of nanomaterials on ALI lung epithelial cells. We retrieved 3 275 hits, from which 140 were identified as eligible for reviewing. A total of 68 studies were finally selected based on the following inclusion criteria: i) the study was conducted on human epithelial lung cells, ii) well-characterized pristine (*i.*e non-modified, non-coated) or standard reference nanomaterials were used, iii) accurate information on cell-delivered dose was available, iv) surface area was provided to be considered as the dose metric and v) cell viability (metabolic activity), LDH cytotoxicity and/or cytokine release were reported. In addition, the GUIDEnano approach (scoring-based system) was used to ensure inclusion of very high or high quality only studies (Fernández-Cruz et al., 2018). Generating particle surface area-based dose-response curves, we investigated acute toxicity effects according to material type. Toxicity ranking based on IC_{50} values from metabolic assays revealed copper and zinc oxides as the most toxic materials for A549 cells. Titanium dioxide was identified as a medium-level toxic material while black carbon was in a low-toxicity regime. Co-culturing A549 cells with differentiated d-THP-1 macrophages tended to mitigate the toxic effects for some materials. Moreover, IL-8 pro-inflammatory secretion showed a similar toxicity ranking as compared to cell viability analysis. Finally, preliminary comparison with selected *in vivo* data on acute pulmonary toxicity indicates good agreement between *in vitro* and *in vivo* toxicity ranking for copper oxide, zinc oxide, titanium dioxide and carbon black materials. On the other hand, MWCNT *in vitro* data did not seem to be representative of *in vivo* response, but the *in vitro* data base is scarce. Overall, relatively simple air-liquid interface cell culture models could be suitable for predicting acute *in vivo* pulmonary toxicity of at least some types of nanomaterials.

Exploratory Application of DMD for Particle Deposition in the Respiratory Tract

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Inhaling dust and particles is an everyday occurrence for humans. However, when these particles contain pathogens, harmful substances, or even therapeutic agents, it becomes essential to understand how and where they are deposited in the respiratory tract. In particular, the spatial distribution of radioactive particles is of great concern, as it informs us about which organs are at risk and the radiation dose they might receive. Moreover, understanding and predicting particle accumulation in the airways is crucial for informing other models related to human exposure and intake mechanisms.

Simulating the complex geometry of the respiratory tract and the intricate interactions between airflow and particles requires significant computational resources. To reduce the computational burden, we explored the potential of using Dynamic Mode Decomposition (DMD) as a reduced-order model to infer the trajectories of particles inhaled during a breathing cycle. DMD's strength lies in its ability to distill system dynamics, which we aimed to leverage to predict particle movement.

In our study, we tested various mathematical architectures and data organization methods for applying DMD and compared the results to a high-fidelity computational fluid-particle dynamics (CFPD) simulation. While DMD effectively reconstructs fluid fields, preserving mean flow regimes, it exhibited some deviations when applied to Lagrangian particle fields, particularly in terms of exact spatial deposition. However, the method performed well in capturing the overall distribution of particles, providing accurate insights into how particles spread across different regions of the lungs and trachea.

Although DMD may not precisely predict individual particle locations, it proves valuable in representing the general distribution patterns, preserving the profile shapes, and offering a computationally efficient alternative for particle deposition analysis.

Title: **Dosimetry and dose metric considerations on in vitro inhalation toxicity testing**

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Recently, new approach methods (NAM) have been utilized to reduce animal experimentation and housing and care costs. The use of NAM to assess safety is paramount, underscoring the need for the method to be established for validity and reliability. NAM for inhalation toxicity is now developing to replace traditional animal inhalation toxicity tests. To align with animal inhalation toxicity studies, dose metrics for in vitro inhalation must be consistent with those of animal studies. In this study, we attempted to study the key elements of dose metric characterization in vitro inhalation toxicity test based on an air-liquid interphase (ALI) system. NaCl nanosized aerosols and protein aerosols were generated and exposed to 6-transwell ALI. We studied appropriate exposure dose metrics for in vitro inhalation study such as particle size, number, maintenance of concentration during exposure period, and deposition variability during two hours of exposure period. Our in vitro dose metric study demonstrated compatibility with in vivo dose metrics, aligning with regulatory inhalation guidelines.

Keywords: In vitro inhalation, New approach methods, Air-liquid interphase, Dosimetry, Dose metric

In Silico Modeling to Support Development and Approval of Generic Orally Inhaled Drug Products in the United States

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In the United States, generic drug products rely on the safety and efficacy of the reference listed drugs (RLDs). Establishing bioequivalence (BE) is important to support therapeutic equivalence of the generic products to their respective RLDs prior to product approval. For locally acting orally inhaled drug products (OIDPs) that include dry powder inhalers (DPIs), solution-based metered dose inhalers (MDIs), and suspension-based MDIs, BE is typically demonstrated via a combination of in vitro and in vivo BE studies. Recently, recommendations in new product-specific guidance (PSG) documents for OIDPs posted by the U.S. Food and Drug Administration (FDA) have included information on the use of in silico modeling by applicants to help facilitate generic OIDP development and approval. These in silico methods include regional lung deposition modeling via semi-empirical or computational fluid dynamics (CFD) methods, as well as physiologically based pharmacokinetic (PBPK) modeling. To assist applicants with construction of useful models, the PSG recommendations include information on model purpose, lung region definition, establishment of model credibility, and statistical comparisons. These recommendations are based on FDA-funded external and internal research programs that were initiated with the intention to both assist applicants with the development of fit-for-purpose models as well as to assist FDA with PSG recommendations for relevant in vitro and in vivo studies. Current research focuses on CFD modeling for DPIs and MDIs, in vivo imaging methods to provide validation data sets for regional deposition models, PBPK model structure enhancement, and in vitro permeability and dissolution testing to provide PBPK model parameters. This presentation will provide details on new PSG recommendations for OIDPs that concern in silico modeling, currently active external and internal research, and current research gaps.

Stochastic Modeling of Radionuclide Inhalation Dosimetry for Optimized Radiation Countermeasures and Consequence Management Applications

Emmanuel M. Mate-Kole, Shaheen A. Dewji, Georgia Institute of Technology, Atlanta, GA

Releases from nuclear or radiological security events may result in significant internal radiation contamination through inhalation of particulate contaminants, given the prolonged suspension of particles in the air post-event. Assessing internal radiation dose relies heavily on biokinetic models that determine radionuclide biodistribution over time. The International Commission on Radiological Protection's (ICRP's) human respiratory tract model (HRTM) is widely used for inhaled radionuclides, providing reference models for dose coefficients. However, capturing population-specific variability requires stochastic modeling.

The study aimed to evaluate the variability of deterministic biokinetic/dosimetry models in representing the stochastic nature of radionuclide metabolism in a non-reference population exposed to realistic source terms, using the updated HRTM from the ICRP Publication 130. The complexities of the ICRP particle deposition (PD) model were deciphered and reconstructed into an independent computational module and integrated into REDCAL, an in-house Python-based radiological exposure dose calculator. REDCAL has since been expanded for stochastic analysis, incorporating HRTM deposition components and applying probability distribution functions to uncertain parameters through Latin Hypercube Sampling.

Comparison with reference data for total PD fractions in a reference worker, a nose breather, across activity median aerodynamic diameters from 0.3 μm to 20 μm, showed a 1.04% relative and 0.7% absolute difference, indicating good agreement with ICRP deposition fractions. However, when incorporated military respiratory physiological conditions based on short-term activity budgets, PD fractions showed a maximum relative difference of 70%, leading to significant differences in the committed effective dose. Stochastic analysis for realistic occupational radionuclide intake was then conducted leveraging machine learning models, considering a lognormal particle size distribution with a median of 5 μm. Uncertainty quantification and parameter sensitivity across various radionuclides will be discussed. This study offers a unique stochastic perspective on inhaled particulate metabolism, enhancing radiation consequence management, medical countermeasures, and dose reconstruction for epidemiological studies.

Modernizing Workflows for Environmental Inhalation Risk Assessment: Challenges and Research Needs to Support Coherent Evidence Integration

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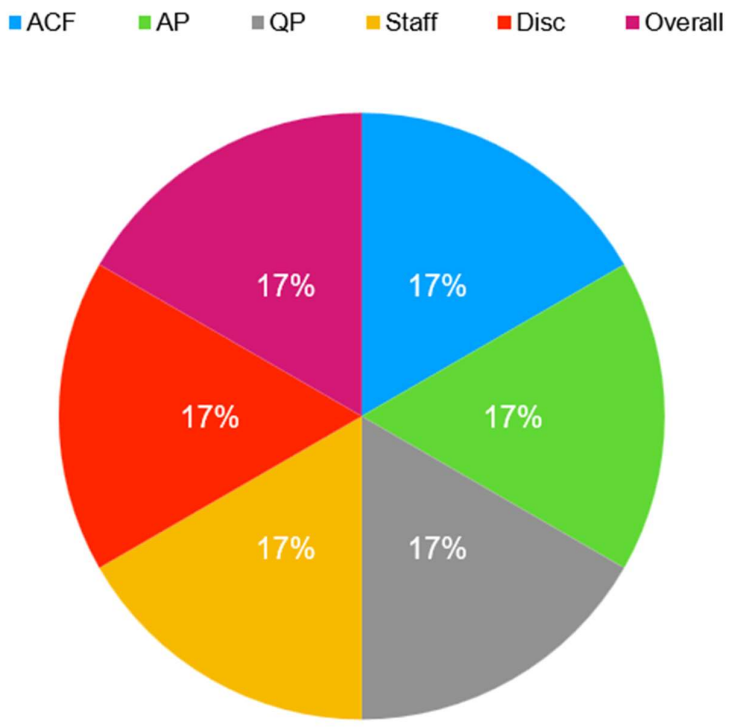
Inhaled environmental substances may cause portal-of-entry effects in the respiratory tract or enter the systemic circulation. Evidence integration for inhalation risk assessment at the US EPA for hazardous air pollutants and criteria pollutants has been historically challenging due to interspecies differences in anatomy and physiology that interact with the various physicochemical (PC) properties of these inhaled agents (aerosols, reactive gases, volatile organic chemicals or VOC) to impact deposition mechanisms and alter resulting uptake and toxicity. While novel approach methods (NAMs) using in vitro test systems are a rapidly emerging area for toxicity testing, such integration challenges persist for NAMs, so both exposure and effect measures must be carefully characterized to incorporate these new data. Development of transparent workflows for collecting, aligning, and integrating data from diverse experimental platforms (e.g., human clinical or epidemiological studies, *in vivo* animal studies, NAMs) is critical to ensure information is used effectively, credibly, reliably, and transparently. Coupling the adverse outcome pathway (AOP) that organized data on key events of pathogenesis and the analogous aggregate exposure pathway (AEP) framework provides a source-to-outcome continuum that enables risk-based, hazard-based, or exposure-based decision making. Multi-scale dosimetry models are thus now necessary to provide quantitative description of interactions among exposures, **PC properties, key anatomical and physiological features, and major mechanisms of ADME (absorption, distribution, metabolism, and excretion) in the respiratory tract to characterize the target site exposure (TSE) and aid both interspecies and in vitro to in vivo extrapolation (IVIVE). The role of template dosimetry models and expert peer review in strategic development of** integrated approaches to testing and assessment (IATAs) and how EPA is modernizing it assessment workflows are featured. Specific data needs and a call for data reporting standards based on the FAIR (findable, accessible, interoperable, reusable) principles for refinement and impactful regulatory application are also discussed.

IV. EVALUATIONS

RATING SCALE				
Poor	**Fair**	**Average**	**Good**	**Excellent**
1	**2**	**3**	**4**	**5**

	Average	Std. Deviation
Adequacy of Conference Facility	4.9	0.3
Adequacy of Program	4.5	0.5
Quality of Program	4.8	0.4
Helpfulness of Conference Staff	4.9	0.3
Adequacy of Discussions	4.5	0.7
Overall Evaluation	5	0.0

One hundred present of those responding, answered "Yes" to the question, "Would you like to attend future similar conferences?"



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Advances in Aerosol Dosimetry Research Conference
October 16-18, 2024

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