

Meeting Reports for 2013: Recent Advances in Breath Biomarker Research

Joachim D. Pleil¹, Wolfram Miekisch², Terence H. Risby³,
Michael C. Madden⁴ and Jon R. Sobus¹

¹National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

²Department of Anaesthesia and Intensive Care Medicine, University Hospital of Rostock, Rostock, Germany

³Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA

⁴National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

This article reports the efforts of the breath research community affiliated with the International Association of Breath Research (IABR) in disseminating research results in high profile technical meetings in the United States (US). Specifically, we describe presentations at a new venue for us at meetings of the Society of Toxicology (SOT - <http://www.toxicology.org/>), and our growing presence at the Pittsburgh Conference and Exposition, "PittCon" (<http://www.pittcon.org/>) that we have reported on in previous years (Pleil 2010 and 2011, Pleil et al. 2012). We have also commented on our own IABR meetings (Amann 2009, Dweik 2011, Pleil and Amann 2011, Corradi and Mutti 2011) as well as a related meeting of the Submarine Air Monitoring and Air Purification (SAMAP) community (Pleil and Hansel 2011). The hope is that we can disseminate current thought and outreach of the IABR community.

The present article is limited to breath related topics in SOT and PittCon meetings in 2013. We encourage the readership of the Journal of Breath Research (JBR) to contribute their own thoughts and observations from other related meetings and workshops to the editors for inclusion in future reports. We note that most names mentioned in the following text are researchers associated with IABR and JBR.

Society of Toxicology, 2013 Meetings

The SOT is a research organization dedicated to the "...*study of the adverse effects of chemical, physical, or biological agents on people, animals, and the environment.*"; the annual SOT meeting hosts about 7,000 attendees and the society also arranges smaller

specialty meetings (approx. 200 – 300 attendees) based on their regional organizations. SOT represents a potentially important new scientific community for implementing breath based methodology in that our concept of “non-invasive monitoring” has value for longer term longitudinal studies, both in animal and human toxicological research. This year, we participated in the annual SOT meeting in San Antonio, Texas and we also contributed to the SOT regional specialty conference co-hosted by US Environmental Protection Agency (EPA) in Research Triangle Park, North Carolina.

Symposium/Workshop: “Pulmonomics, the Exposome, and Microbiomes in Immunotoxicology”

Dr. Joachim Pleil from the U.S. Environmental Protection Agency (US EPA) (pleil.joachim@epa.gov) arranged this workshop for the SOT Annual meeting. The session was sponsored by the Immunotoxicology Specialty Section with endorsements from Biological Modeling, Clinical and Translational Toxicology, and Inhalation and Respiratory Specialty Sections. The session was aligned with the SOT theme “Biomarkers for Exposure Assessment, Safety Evaluation, and Translational Medicine”. An introductory presentation by Dr. Michael Madden from US EPA (madden.michael@epa.gov) discussed the growing evidence for the role microbes play in determining responses to toxicants and in the development of disease, which could be studied via biomarkers of the exposome and pulmonomics. All of the subsequent workshop speakers presented aspects of exogenous chemical interaction with the mammalian exposome featuring pulmonary effects.

Specifically, Dr. Madden and Dr. Jon Sobus (sobus.jon@epa.gov) from US EPA presented invited talks featuring aspects of breath analysis that fit into the focus of this workshop. Dr. Madden introduced his findings with data demonstrating improved methodology for collecting substances in human breath for subsequent analyses. Water vapor and associated constituents in human exhaled breath were collected in a more reproducible manner via use of a computer-controlled metronome to dictate breathing patterns (e.g., breathing, tidal volume) with subsequent condensation of the liquids. Breath proteins were able to be collected via just a few exhalations onto/through disposable filters. The detection of these trace proteins was made possible by improved commercial enzyme-linked immunosorbent assay (ELISA) technology using a final detection step of electrochemically-induced chemiluminescence. The filters also could be cultured for microbial content and subsequent volatile organic compounds (VOC's) profiles examined to determine possible unique markers of aerobic and anaerobic organisms. Findings to date suggested inter- and intra- human variability. Time of flight mass spectrometry (ToF MS) detection with automated discovery features for identifying major differences in peaks was deemed useful for tentative peak identification and validating the specificity of biomarker profiles.

Dr. Sobus introduced biomarker research efforts within EPA's Chemical Safety for Sustainability (CSS) research program (<http://epa.gov/research/docs/css-strap.pdf>). Key objectives of the CSS program are to develop tools for rapid screening and prioritization

of environmental chemicals, and to understand real-world risk from chemicals. Here, breath-based biomonitoring is a useful *in vivo* tool for evaluating the breadth of chemical exposures, as well as physiological responses to exposure events. As such, measurements of human breath provide a useful complement to results from *in vitro* toxicity experiments and *in silico* predictions. Dr. Sobus highlighted that the same measurements tools, including those used to monitor breath, are well-suited for both targeted and untargeted research efforts, and encouraged investigators to identify opportunities for research integration.

Other lectures involved the lung allergenic response to household dust being modified by the presence of an indoor/outdoor dog using a rodent model of allergy; a description of a unique set of airway and nasal transcriptome used to prospectively predict tobacco related lung cancer development risk not only in smokers, but potentially in those exposed to secondary tobacco smoke; and a comprehensive survey of immune-mediated adverse effects of drugs and environmental agents focusing primarily on adverse idiosyncratic reactions. On the whole, this workshop gathered a wide array of pulmonary research topics all of which employ forms of exhaled breath and/or pulmonary/nasal fluids analysis. We also note that Prof. Gunther Oberdorster (University of Rochester) was rewarded for his long and active pulmonary research career examining the health effects of air pollutants with the Lifetime Achievement Award presented by the Inhalation and Respiratory Specialty Section.

Specialty Meeting: "Exposure: A Forgotten Part of Toxicology?"

In addition to various student poster and platform presentations, this meeting featured four plenary lectures covering aspects of the relationships among chemical exposures, biological stressors and the resulting internal toxicological doses. One of these lectures was presented by Dr. Pleil wherein he discussed the exhaled breath applications for toxicological modeling focusing on the use of exhaled breath aerosols analyses for proteins and other large molecules. Here, the new concepts for immunochemistry based breath analysis and the implementation of data visualization via heat-mapping recently published in JBR (Pleil et al. 2011) were featured.

PittCon 2013, Technical Sessions

As mentioned above, we (IABR members) have participated in a number of recent PittCon meetings. Briefly, PittCon is an annual International meeting focusing on analytical chemistry methods, tools, and instrumentation. PittCon typically attracts about 20,000 attendees and 1000 commercial exhibitors. This year the conference was held in Philadelphia, Pennsylvania from March 16-21. Over the past few years, breath analysis science has taken on an ever-increasing role at PittCon meetings. Once again, breath analysis was featured in an invited symposium, in a conferee networking session, and invited technical lectures in various additional sessions.

Symposium: “Human Exposome Discovery and Disease Investigation”

This session was organized by Dr. Pleil and Dr. Wolfram Miekisch (wolfram.miekisch@uni-rostock.de) from University Hospital of Rostock Germany; it was comprised of different aspects of exposure assessment and clinical methodologies incorporating breath analysis. The topics ranged from the general philosophical to the detailed technical. After a brief overview by Pleil, the first two presentations by Prof. Terence Risby (thrisby@jhmi.edu) of Johns Hopkins University and Prof. Jochen Schubert (jochen.schubert@uni-rostock.de) from University Hospital of Rostock Germany, respectively, revolved around the broader issues of medical diagnostics wherein both speakers cautioned about using specific breath biomarkers in case-control studies for predicting disease state. The general consensus of these two presentations was that cross-sectional or screening type analyses may not be probative due to large variance for all biomarkers among subjects. However, all hope is not lost; specific biomarkers can and should be used to track within person changes in response to treatment or in disease progression as each individual serves as his or her own control. Prof. Cristina Davis (cedavis@ucdavis.edu) subsequently presented some of her group’s engineering work in developing specialty surfaces for collecting exhaled breath condensate (EBC) without external cooling designed to operate in situations where cryogenics would be impractical. The methodology is based on formation of microdroplets on hydrophobic surfaces that have specific patterns of nano-scale ridges that encourage the growing droplets to a central collection point via force created by surface tension. Dr. Stephen Edwards (edwards.stephen@epa.gov) from US EPA described a complex US EPA study of the environmental and genetic linkage for human pulmonary disease focusing on asthma. The study encompassed a wide array of tests including breath odor and VOCs analyses and pulmonary function tests serving as pre-clinical effects. The final presentation was by Dr. Michael Phillips (mphillips@menssanaresearch.com) from Menssana Research, Inc., Newark NJ, USA who gave an overview of the breath-testing paradigm from a commercialization aspect. He also presented a series of data interpretation techniques focusing on his recent work as to how the exhaled breath exposome is affected by radiation exposure in humans. We note that Dr. Phillips has recently been honored with a special issue of JBR (see Risby and Pleil 2013) and that this lifetime achievement was recognized by the speakers. In summary, the session was quite lively with high-level audience interaction after each presentation.

Conferee Networking: “Non-invasive biomedical analysis: of muscles and men – breath testing for metabolic monitoring”

These sessions are designed to provide a less formal venue for discussing current topics within a broader context. This year, the breath session was led by Dr. Miekisch and Prof. Schubert and revolved around metabolic monitoring by means of breath biomarkers. The session started with pros and cons of diabetes assessments via different forms of exhaled breath analysis. The ultimate outcome of these discussions was that traditional acetone (and other related biomarkers of metabolic disturbance) measurements might not be sufficient for cross-sectional diagnostic evaluations and do not mirror blood glucose

levels. This is demonstrated by the large within and between patient variances even in “unremarkable” patient groups that have no diagnosed metabolic diseases. As concentrations of related breath markers such as acetone are affected by more than one biochemical pathway, follow up studies of relative changes /within person changes) in patients are much more promising to understand underlying physiology and biochemistry (e.g. Miekisch et al. 2012). For this purpose fast and continuous measurement methods such as PTR-MS but also highly specific sensors are preferable. Promising examples from measuring metabolic changes during exercise were discussed. The participants concluded that breath biomarkers bear a huge potential for metabolic monitoring. Before breath biomarkers can be used for diagnostic purposes, origins, biochemical pathways, effects of patho-physiology as well as clinical requirements must be taken into account.

Other technical breath related presentations and technology:

Additionally, “Recent advances of Needle-Trap-Microextraction (NTME) for in vivo breath analysis” was presented by Phillip Trefz (phillip.trefz@uni-rostock.de) from University Hospital of Rostock Germany. He focused on automation of breath sampling and effects of sampling parameters on sensitivity and reproducibility. As NTME is affected by humidity, sampling flow and specific properties of the adsorption materials, thorough control of sampling procedures is mandatory for in vivo application of NTME. In conclusion, triple bed NTME rather than single bed devices are preferable for in vivo sample preparation. The newly developed CO₂ controlled automated sampling device combines the control of relevant sampling parameters with easy and fast sampling. Juliane Obermeier (juliane.obermeier@uni-rostock.de) from University Hospital of Rostock Germany gave a presentation that described an electrochemical sensor system for detection of volatile aldehydes and inflammation markers in breath. She concluded that portable sensor systems provide optimal conditions for non-invasive and point-of-care analysis of volatile aldehydes and inflammatory markers in the ppbv range and cautioned that for clinical application, specific sensitivity of the sensors and confounding variables have yet to be defined. At the industrial exhibition more than 1000 companies from 28 countries presented actual instruments and new developments. A large number of visitors were attracted by the chance of experiencing real-time breath analysis at the Ionicon Analytik booth by means of a proton transfer reaction quadrupole mass spectrometer (PTR-QMS) instrument connected to a buffered end tidal (BET) breath sampling inlet. Dr. Jens Herbig (jens.herbig@ionimed.com) and Dr. Lukas Mark (lukas.mark@ionicon.com) of Ionicon also presented at the poster sessions wherein PTR instruments applications to gas analysis were featured.

Summary

Overall, the IABR community’s penetration into the toxicology and analytical instrumentation arenas via SOT and PittCon technical meetings was successful. The breath researchers were well received by members of these disparate disciplines and we hope to continue to pursue such meetings to spread our experience and the valuable

contributions of breath biomarkers measurements. We especially encourage our members to participate in meetings like PittCon and SOT, and anticipate proposing and hosting new sessions in future years. As always, we continue to encourage our PittCon colleagues to participate in breath-specific endeavors by reading JBR, by scanning the IABR website, and possibly attending the next IABR meeting.

References

Amann A, 2009. “Breath 2009: International conference on breath and breath odor research (26-30 April 2009, Dortmund, Germany)”, *Journal of Breath Research*, 4:01021 (1 pp).

Corradi M and Mutti A, 2012. “News from the Breath Analysis Summit 2011”, *Journal of Breath Research* 6:020201 (6pp).

Dweik RA, 2011. “The great challenge for exhaled breath analysis: embracing complexity, delivering simplicity”, *Journal of Breath Research* 5:03021 (4pp).

Miekisch W, Herbig J and Schubert JK, 2012. “Data interpretation in breath biomarker research: pitfalls and directions”, *Journal of Breath Research*, 6:036007 (10 pp).

Pleil JD, 2010. “Meeting Report: Breath Biomarkers Networking Sessions at PittCon 2010, Orlando Florida”, *Journal of Breath Research*, 4:029001 (5pp).

Pleil JD, 2011. “Meeting Report: Breath Biomarkers Networking Session at PittCon 2011”, Atlanta, GA., *Journal of Breath Research* 5:029001 (4pp).

Pleil JD and Amann A, 2011. “The 2011 International Association of Breath Research (IABR) meeting in Parma Italy: A collection of comments from attendees”, *Journal of Breath Research* 5:049001 (5pp).

Pleil JD, Stiegel MA, Sobus JR, Liu Q, and Madden MC, 2011. “Observing the human exposome as reflected in breath biomarkers: heat map data interpretation for environmental and intelligence research”, *Journal of Breath Research* 5:037104 (9pp).

Pleil JD and Hansel A, 2012. “Submarines, spacecraft and exhaled breath”, *Journal of Breath Research* 6:019001 (3pp).

Pleil JD, Stiegel MA, Kormos TM, and Sobus JR, 2012. “Breath analysis science at PittCon 2012, Orlando, Florida”, *Journal of Breath Research* 6:039001 (6pp).

Risby TH and Pleil JD, 2013. “Breath analysis—past, present and future: a special issue in honor of Michael Phillips’ 70th birthday”, editorial: *Journal of Breath Research* 7:010201 (3pp).

Acknowledgements

The authors are thankful for thoughtful advice and comments from the various participants, audience members, and colleagues from IABR, PittCon and SOT meetings. The United States Environmental Protection Agency through its Office of Research and Development has subjected this article to Agency administrative review and approved it for publication.