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Report: New analytical and statistical approaches for interpreting the relationships among environmental stressors and biomarkers

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Overview:

The broad topic of biomarker research has an often-overlooked component: the documentation and interpretation of the surrounding chemical environment and other meta-data, especially from visualization, analytical, and statistical perspectives (Pleil et al. 2014; Sobus et al. 2011; Pleil et al. 2011). A second concern is how the environment interacts with human systems biology, what the variability is in "normal" subjects, and how such biological observations might be reconstructed to infer external stressors (Sobus et al. 2010; Pleil and Sheldon 2011; Pleil 2009; Tan et al. 2012; Zhu et al. 2013a; Bean et al. 2014a). In this article, we report on an eclectic collection of recent research presentations from a symposium at the 248th American Chemical Society meeting held in San Francisco, August 10 - 14, 2014, that focused on providing some insight into these important issues, particularly, approaches and impacts on our understanding of biomarker data. The symposium, organized by the authors of this report, was comprised of both posters and platform presentations. The intent of this symposium was to address the observation that many of the novel scientific approaches being used to explore the effects of environmental stressors on human health and well-being are creating information faster than can be properly analyzed across studies. In order to reach a broad ACS audience, a call was sent out entitled "Monitoring and evaluating environmental exposures: Scientific case studies incorporating statistical approaches to evaluate and predict from large and fuzzy data sets," with a focus on evaluating data that could be used to assess the ultimate pathways from the environment to internal dose and allowing for more specific case studies that would reflect current research trends. The selected presentations included environmental measurement studies delineating exposures as well as studies linking biological measurements to an environmental source. In aggregate, these presentations served the purpose of answering different questions regarding the human exposome, which is directly impacted by the interaction with the environment (Rappaport and Smith 2010, Pleil 2012). Here, we describe the major concepts explored in the presentations and put them into a common framework for informing the overall field of biomarker analysis.

Environmental Measurements

In order to identify biomarkers for the health effects of environmental exposures, it is necessary to make relevant and accurate measurements of the chemical or biological agents in the environment. There are thousands of contaminants of emerging concern (CECs) to monitor and due to constraints on resources (e.g., funding, instrument time, et cetera), prioritization is required. Dr. Arjun Venkatesan and Prof. Rolf Halden of the Biodesign Institute at Arizona State University are employing sewage epidemiology and sewage metrology approaches to survey the spectrum of CECs that humans consume and to which they are exposed (Venkatesan and Halden 2014a). During the symposium, Venkatesan presented a study in which he and Halden screened 231 CECs in samples from the National Sewage Sludge Repository, the largest archive of municipal sewage sludge (MSS) in the nation, comprised of samples from 164 wastewater treatment plants from across the US (Venkatesan et al. 2014b). They found that the most abundant class of CECs in the MSS were alkylphenols and their ethoxylates, followed by pharmaceuticals and personal care products, then brominated flame retardants, and that abundances were proportional to population usage. The authors compared the abundance of a subset of these MSS CECs (52 of 231) to nationally-representative human biological specimens, revealing a 70% overlap. Therefore, they propose measurement of CECs in MSS as a means to quantify ongoing exposures, and to use sewage metrology data to monitor and prioritize those CECs most likely posing a risk to human health.

Not only are there many thousands of primary pollutants that can impact human health, but these compounds can undergo chemical transformations or react with each other, producing toxic secondary pollutants. Prof. Sibel Menteşe in the Department of Engineering at Çanakkale Onsekiz Mart University in Turkey is investigating these reactions in indoor environments. During the symposium she presented the work that she and her colleagues are conducting on the formation and dispersal of secondary organic aerosols (SOAs), volatile organic compounds (VOCs), and nano-scale particles from reactions between indoor ozone and terpenes. In order to model and measure these reactions, they used a test room – complete with standard building materials and furniture – in which they dose ozone and measure the subsequent formation of SOAs by reactions with terpenes and other primary pollutants released by the room contents. They found that the concentrations of the secondary pollutants increase rapidly with ozone-dosing, and the products were slow to disperse from the indoor environments. They propose that more studies need to be done to identify and characterize secondary pollutants, especially in indoor environments, where exposure could potentially have a significant impact on human health.

Once environmental contaminants have been targeted for monitoring, how they are measured must be considered. For instance, making *in situ* measurements in the environment requires highly specific and selective detection tools, while *ex situ* analyses require procedures for acquiring representative samples, which were addressed in presentations by Ms. Jennifer Apell and Prof. Heather Hunt, respectively. Hunt's lab in the Department of Bioengineering at the University of Missouri is focused on developing and characterizing nanostructured materials for environmental monitoring devices,

including the detection of food and waterborne pathogens in aqueous environments. Part of her focus is to improve the specificity of a unique type of optical biosensor, the Whispering Gallery Mode (WGM) microcavity, by functionalizing the surface with pathogen-specific recognition elements (Hunt 2014). During the symposium, she reported on her progress toward developing highly-specific WGM sensors for the detection of *H. pylori*, with current detection limits on the order of 10^4 cells/mL, as well as WGM sensors specific for chitin (Dahmen 2014), developed for the universal detection of two major threats to crops: fungal pathogens and insects. Apell, a graduate student in the lab of Prof. Philip Gschwend in the Department of Civil and Environmental Engineering at MIT, is developing new sampling methods for measuring freely-dissolved concentrations of persistent organic pollutants (POPs) at Superfund sites in order to generate better estimates of POP concentrations to which aquatic animals are exposed, and thus, their toxicity. Apell evaluated the performance of *in situ* passive samplers for measuring freely-dissolved polychlorinated biphenyl (PCB) concentrations versus standard estimates using sediment concentrations. One important result was that the *in situ* samplers provided more reproducible values for pore water PCB than the estimates from sediment concentrations, which is an important factor in predicting exposure, and by extension, for identifying biomarkers of toxicity, cleanup thresholds, and bioavailability.

Measuring the impact on humans

One of the difficulties of quantifying human exposures to toxic compounds is that they are often metabolically-modified into multiple metabolic and anabolic chemicals with different bioaccumulation properties. Thus, identifying an appropriate biomarker of the parent compound can be challenging. Ms. Wen Xin Koh, a graduate student at the University of Iowa, who is co-advised by Profs. Keri Hornbuckle in Civil and Environmental Engineering and Peter Thorne in Occupational and Environmental Health, is working to quantify as many as 65 hydroxylated polychlorinated biphenyls (OH-PCBs) in human serum. OH-PCBs are common metabolic breakdown products of PCBs and serve as biomarkers of PCB exposure. Importantly, some of the OH-PCB congeners are reported to be more toxic than their parent compounds. During the symposium Koh presented a study of adolescents and their mothers in the industrial area of East Chicago, IN and rural Columbus Junction, IA, in which she hypothesized that the sera of adolescents would have significantly lower amounts of OH-PCBs than their mothers, and that the congener profiles would differ. She and her colleagues found that there was a significant difference in the sum of OH-PCBs between generations in Columbus Junction, but no statistical difference between generations in East Chicago. They were able to detect 55 of the 65 congeners in serum, and, importantly, the lower chlorinated OH-PCB congeners were undetectable. They hypothesize that these compounds are being metabolized further, and therefore PCB metabolites other than OH-PCBs, may serve as better biomarkers for lower chlorinated PCB congeners.

Linking environmental and human data to identify biomarkers

Identifying biomarkers of environmental exposures requires the collection and synthesis of large datasets, both from the human and the environmental sides of the

equation. During the symposium, Prof. Menteşe described an ambitious collaborative project between the Departments of Environmental Engineering, Medical Microbiology, Public Health, and Respiratory Medicine at Çanakkale Onsekiz Mart University that is attempting to identify associations between respiratory problems and air pollution in three locations around Çanakkale, Turkey. The study, which is in progress, is collecting one year of data, at monthly intervals, from over 1,300 human subjects and 150 environmental sites. The data include measures of human health – e.g., respiratory function (FEV_1/FVC ratio) and health survey data – and indoor and outdoor air quality, characterized by VOCs, bioaerosols, particles, CO_2, CO, ozone, as well as meteorological factors such as temperature and relative humidity. Although the synthesis of the data is in its initial stages, Menteşe reports that they are observing significant seasonal and spatial variations for indoor and outdoor air quality, and that fatigue is the most prevalent symptom related to indoor air quality among the healthy participants.

Knowing how to begin to make sense of such large data sets is a daunting challenge. One method is to take a reductionist approach, using model systems to identify putative biomarkers related to key components of the environment-human interaction. Dr. Heather Bean and her colleagues in the lab of Prof. Jane Hill in the Thayer School of Engineering at Dartmouth College are working on developing breath-based diagnostics for lung infections and exposures. They have found that breath biomarkers from murine model systems are specific and selective enough to identify the etiology of lung infections down to the level of bacterial strain (Zhu, et al., 2013a; Zhu, et al., 2013b) and bacterial mutations (Bean, et al., 2014b). In the work presented during the symposium, they hypothesized that the pathogen-specific biomarkers are not only arising from the bacteria, but also from the host's specific response to the *Staphylococcus aureus* or *Pseudomonas aeruginosa* antigens. Through the application of the supervised statistical method partial least squares-discriminant analysis (PLS-DA), they confirmed that the host is producing breath volatiles that are specific to, and predictive of, the antigen source (Bean, et al., 2014a).

Dr. Joachim Pleil from the US Environmental Protection Agency (EPA) Office of Research and Development presented another approach for making headway into the large and complex datasets of biomarker studies being implemented at the Agency. He focused on the importance of interpretation of measurement (empirical) data in light of surrounding meta-data both in the environmental parameters (location, time, media, sources) and in the human biological parameters (gender, age, ethnicity, phenotype, etc.). Initially, he discussed the current applications of the EPA exposure to health outcome framework and how environment, biomarker, and effects are linked (Sobus et al. 2011) and how *in vivo* biomarker measurements and discovery fit into the larger scheme of environmental health investigations (Pleil et al. 2012, Pleil and Stiegel, 2013). He presented an example from an ongoing EPA study regarding the evaluation of urinary kidney injury panel (KIP) of protein markers using a highly sensitive immunochemistry platform. He showed heatmaps and graphs of KIP data as a visualization tool, statistical methods for comparing data and subject groups, and finally the implementation of multivariate and mixed effects regression models. The presentation demonstrated how visualized data could be used to optimize specific statistical evaluations and to inform future investigations.

Poster presentations

The posters associated with this session had the same eclectic topic diversity. Briefly, they covered projects focused on environmental measurements as well as a mixture of biological measurements combined with other meta-data. The direct environmental studies included a survey of agricultural chemicals in surface waters and radionuclides in the built environment. Another presentation investigated specific links between environmental exposures and resulting chemical biomarkers in biological tissues wherein organophosphate flame-retardants in indoor air particulate matter were found in human placenta samples. Two of the presentations reported results of "discovery" analyses; one presented results from a study of manatees from the Florida coasts wherein they identified waterborne contaminants in manatee blood, and another assessed persistent organic pollutants that bio-accumulate in adipose tissue of Alzheimer's patients. Overall, the contributions to the poster session captured the variety of current environmentally-related studies and served as an excellent complement to the platform presentations.

Summary

All of the submitted projects fit into the general theme of the symposium that environmental and biological measurements are complex, and that we are now in a scientific time where research can no longer be conducted in "silos." To effectively address questions in the area of biomarkers research, we need to broaden the applicability and the methodology for investigating the relationships between the environment and human health. From the symposium, we found that a major component of the evolving research framework involves discovering and documenting as many biomarkers as possible for assessing health states. However, we can never lose sight of the importance of careful collection of environmental measurements that are needed to support and generate insight into public health problems with a biological basis. We also need to consider components of the human exposome beyond chemical biomarkers and include the endogenous biochemicals from human and microbiome metabolism.

As research becomes more and more complex, collaboration among research groups is paramount. It is through such meetings and symposia that we can share concepts, data, and expertise for making comprehensive assessments of the pathway from exposure to health outcome. Furthermore, the authors encourage the readership of the journal to consider engaging in similar research activities, symposia, and other outreach to the biomarkers community and consider writing similar reports.

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