CSS Pathways: Anticipating Impacts of Chemicals
CHEMICAL SAFETY FOR SUSTAINABILITY RESEARCH NEWS

Contents

CSS Highlights

What's in the Dust?

In the field of exposure science, there is a need for rapid monitoring methods to screen for suspected contaminants in the environment. However, our current arsenal of data and scientific approaches are unlikely to meet this demand. . To advance the science, CSS researchers utilized "non-targeted" and "suspect screening" analyses, in which samples are analyzed without a hypothesis driven list of selected chemicals. In the novel work, *Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring*, CSS researchers integrated high-resolution mass spectrometry (HRMS) data and exposure and toxicity data (using ExpoCast and ToxCast) for chemical prioritization.

In this study, household dust samples (from the American Healthy Homes Survey) were screened using liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS). Chemicals were validated using unique molecular features and standards, and compared to a chemical database. They were then prioritized based on abundance and frequency along with exposure and bioactivity estimates from ExpoCast and Tox21. Some chemicals were ranked as high-priority based on detection frequency, such as piperine, an active ingredient in black pepper. While others were ranked as high priority based on bioactivity, such as triclocarban, a potential endocrine disruptor. Chemical matches in at least 10 of the 56 dust samples include pesticides, flame retardants, and nicotine. Of the 33 total confirmed chemicals more than half were not associated with dust in previous published literature, highlighting the importance of non-targeted screening for the potential identification emerging chemicals.

This seminal work will serve as a framework for future research designed to rapidly identify and quantify emerging unstudied contaminants in media that are relevant to human and environmental health. This work appears in *Environment International*.

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What's in the River?

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have unique physical and structural properties. PFAS are resistant to chemical and thermal degradation and therefore, useful in industrial applications (i.e. stain repellency to textiles). Nevertheless, these same properties also make them extremely persistent environmental contaminants. Manufacturers are phasing out the use and production of long-chain perfluorinated chemistries by substituting alternative shorter-chained materials. However, to date, information about potential fate and toxicities of these alternative chemicals has not been disclosed.

There is little information available about the products replacing traditional perfluorinated compounds. The use of non-targeted screening provides the opportunity to gain additional information on these alternative chemicals. A recently published paper, *Identification of novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in natural waters using accurate mass time-of-flight mass spectrometry (TOFMS)*, was highlighted in an *Environmental Science and Technology* special issue focusing on emerging contaminants in water. CSS researchers and collaborators measured surface water samples from the Cape Fear River, NC where previous sampling efforts showed measurable PFASs. Researchers detected 12 novel perfluoroalkyl ether carboxylic and sulfonic acids in the surface water samples using a non-targeted workflow – time-of-flight mass spectrometry, non-targeted analysis, molecular feature extraction, and evaluation of features unique to the samples. Although more work is necessary to further categorize and determine the extent of contamination of these newly identified chemicals, this methodology holds promise for continued identification and classification of emerging contaminants.

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Searching for a [Nano] Silver Lining

Nanomaterials are increasingly used as key agents in many biomedical and commercial products to discourage the growth of microbes and pathogens. The marked antibacterial properties of some nanomaterials lead to their inclusion in a diverse array of products, from kitchen items to clothing. However, the very same selective pressure induced by nanomaterials within a product may also harm natural bacteria. As the use of nanomaterials increases, it is imperative to understand how these agents might impact humans and the environment. CSS scientists collaborated with scientists in Australia and Italy to publish "*Changes in soil bacterial communities and diversity in response to long-term silver exposure*" in *FEMS Microbiology Ecology*.


In this study scientists investigated the effects of ionic silver (Ag) stress on soil bacterial communities and identified resistant/persistent bacterial populations. The composition and functioning of soil microbial communities are responsible for soil quality through decomposition of organic matter, recycling of nutrients, and parasite control on plants. The study results confirmed that silver (Ag) nanomaterial concentrations remained in soil samples throughout a 9-month incubation period. Doses of silver nanomaterials caused immediate and significant reductions to gene counts within microbial communities, as well as β-diversity changes and increasing α-diversity in response to Ag pressure. The time that microbes were exposed had a stronger influence than the dose rates. These findings will help to better understand soil-based resistomes and how scientists preempt the risks associated between Ag nanomaterials, soil-dwelling bacteria, and clinical pathogens.

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Nanoparticles on the brain?

There are significant challenges and health risks associated with continually emerging engineered nanoparticles (ENM), which are now present in several thousand consumer products. Following inhalation and/or oral exposures, nanoparticles may move from the lungs or gastrointestinal tract to other organs including the liver, kidney, nervous system and even the brain. The conventional research techniques to assess systemic toxicities impacting organs are expensive, time consuming, and require significant numbers of animals. The U.S. EPA identified the development and use of rapid and translational alternative approaches for multiple organ nanoparticle hazard assessment as a critical need.
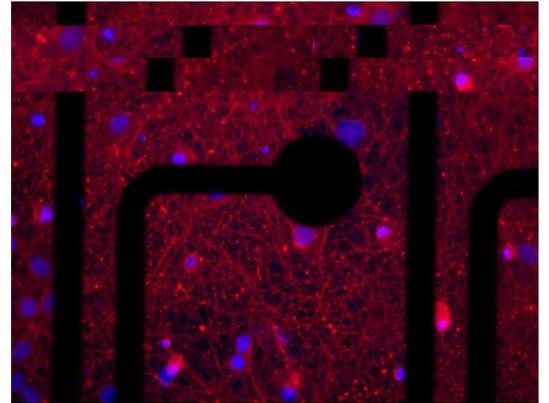
A study conducted by CSS researchers recently published in *Nanotoxicology*, *In vitro screening of metal oxide nanoparticles for effects on neural function using cortical networks on microelectrode arrays,* used higher throughput methods to screen and prioritize nanoparticles for additional testing. Five cerium oxide and four titanium oxide nanoparticles of varying sizes were examined on network function in primary cultures of rat brain cortex on 12 well microelectrode array (MEA) plates. *In vitro* MEA methods use a grid of independent extracellular microelectrodes to record cellular electrical activity. These methods are already used to characterize the neurotoxicity/neuroactivity of a broad classes of drugs and chemicals. The results of this study indicate that some metal oxides particles cause subtle concentration related changes in spontaneous and/or $GABA_A$ receptor mediated neuronal activity in the absence of cytotoxicity. $GABA_A$ is the chief inhibitory neurotransmitter which sends chemical signals between the nerves responsible for body function. Additionally, results prove MEAs can be used to screen and prioritize nanoparticles for neurotoxicity hazard, an important step in the use of high throughput methods for emerging chemical evaluation.

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Defining and Refining Adverse Outcome Pathways

Risk assessors today face increasing demands to assess more chemicals, with greater speed and accuracy, and to do so using fewer resources and experimental animals. New approaches in biological and computational sciences provide mechanistic information that could help in meeting these challenges. Adverse Outcome Pathway (AOP) provides a conceptual construct that organizes existing knowledge about the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment.

Two recent publications describe the evolution of the AOPs and demonstrate how incorporation of additional knowledge on chemical exposure, along with absorption, distribution, metabolism, and excretion (ADME) will improve chemical safety decisions.

The mini-review titled, _Adverse Outcome Pathways – Organizing Toxicological Information to Improve Decision Making,_ was highlighted as the cover article for the January issue of the _Journal of Pharmacology and Experimental Therapeutics_. The manuscript provides an overview on the state of the science regarding AOPs. The authors describe the intersections and subtle distinctions between AOP and mode of action (MOA) analyses for supporting environmental regulatory decisions. On-going and future applications of these concepts for developing Integrated Assessment and Testing Approaches (IATA) used to support regulatory applications are discussed.

Another publication from January in _Environmental Health Perspectives_, _A Workflow to Investigate Exposure and Pharmacokinetic Influences on High-Throughput In Vitro Chemical Screening Based on Adverse Outcome Pathways_, demonstrates the utility of augmenting the AOP framework through the incorporation of exposure and ADME considerations to improve toxicity predictions. As a case study, CSS scientists applied this enhanced workflow using existing ToxCast HTT data and AOP for acetylcholinesterase (AChE) inhibition. The study highlights the importance of incorporating exposure and ADME properties in refining the interpretation of HTT data. This workflow can improve the reliability of _in vitro_ data by identifying "false positives" (e.g. chemicals with no exposure potential) which, ultimately, reduces the time and cost spent on additional chemical-specific risk analyses.

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NPD Corner

Happy New Year from CSS!

This year brings a few changes to the CSS team. Our Deputy National Program Director Elaine Cohen Hubel is on a detail as Acting Division Director at NHEERL/ISTD and John Cowden has joined as the Acting Deputy in the interim. John will also maintain his role as Matrix Interface for CSS and National Center for Computational Toxicology (NCCT). John was previously at U.S. EPA's National Center for Environmental Assessment (NCEA), where he was integral on a number of influential reviews including benzo[a] pyrene, inorganic arsenic, and PCBs. He worked as a postdoctoral researcher studying developmental neurotoxicity and holds a PhD in Molecular Biology and Genetics from the University of California at Berkeley. Please join us in wishing Elaine much success and welcoming John.

With our 2016-2019 Strategic Research Action Plan in place, we will continue our implementation phase in 2016. CSS hopes to further increase the visibility and ultimately the use of the cutting edge science produced by U.S. EPA labs and centers. In a recent article in Bloomberg BNA Thomas Burke, the Deputy Assistant Administrator for the EPA's Office of Research and Development, John Bucher, associate director of the National Toxicology Program, and I spoke about the use of emerging science to advance risk analysis. This article highlights several important efforts for this year: updating the iCSS Dashboard to include information on chemical structure and physical chemical properties, developing novel ways to validate high throughput screening data for specific applications, continuing to develop advanced methods to predict human and ecological exposures to chemicals and mixtures, and developing case studies to demonstrate feasibility of high throughput risk assessments. We have an ambitious program that is supported by some of the best researchers in their fields. As Tom said in this interview "We have unprecedented amounts of information that we can combine to help solve problems [and] we're finding quicker, more-efficient ways to do that".

Please enjoy this issue of Pathways and feel free to reach out if you would like to learn more about any of our activities.

Warmly,

Tina

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Upcoming & Recent Events

Society of Toxicology Comes to the Big Easy | March 13-17, 2016

U.S. EPA scientists will showcase their research through symposia, workshops, platform presentations, and poster sessions at the 2016 SOT 55th Annual Meeting in New Orleans, March 13-17, 2015. U.S. EPA will host several special events in conjunction with SOT, including a joint-meeting of U.S. EPA researchers and Science to Achieve Results (STAR) grantees, "*3D or Not 3D: That Is the [Predictive Toxicology] Question…"* to discuss organotypic and three-dimensional cell culture systems on March 17th at 1 - 5pm. U.S. EPA will also provide an exhibit booth to distribute informative research factsheets, career information, and grant opportunities. Once again, U.S. EPA will hold "Meet the Director Sessions" and demonstrations of online research tools at the booth #1351. Information about the SOT Annual Meeting and conference materials are available on the SOT conference webpage.

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NAS Low Dose Meeting | Washington D.C., Feb 3-5, 2016

An expert committee convened by the National Academies of Science (NAS) held its third meeting and a public workshop on the EPA-funded study, "Unraveling Low Dose, Case Studies of Systematic Review of Evidence." The recent workshop brought in a number of experts, including Earl Gray from ORD's NHEERL, to discuss evidence of low dose effect that would help in selection of specific chemicals for potential case studies and systematic review. The overall goal of this committee is to evaluate the evidence of adverse effects from low-dose exposure to endocrine disrupting chemicals and to determine whether EPA regulations are public health protective against this possible route of exposure. More information on the project can be found here.

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Environment and Health: What's the Human Microbiome Have to do with it? | Jan 14-15, 2016

The NAS (National Academies of Science) held a free two-day workshop in Washington D.C. that explored the latest emerging research on microbiome-environment interactions, including how the microbiome could influence responses to environmental exposures, how environmental exposures early in life could change microbiome function and affect later-life health outcomes, and a potential framework to advance research over the next decade. As this is an emerging area of research interest for CSS, Tina Bahadori served on this workshop's organizing committee. More information on this workshop, as well as the recorded presentations can be found here. In addition, ORD and Office of Air and Radiation (OAR) Indoor Air group have collaborated to co-fund with other organizations a new study entitled, *Microbiomes of the Built Environment: From Research to Application*. The first meeting of this committee has been organized for April, 2016. More information can be found here.

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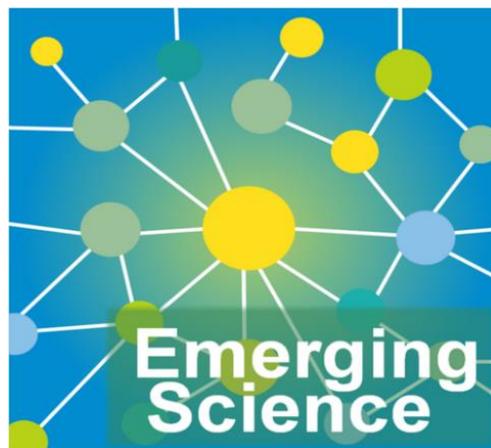
Notables

Metabolism Challenge Launches

In January the U.S. EPA, along with the National Institutes of Health's National Center for Advancing Translational Sciences, and the National Toxicology Program launched the [Transform Tox Testing Challenge: Innovating for Metabolism](#). Current high-throughput assays do not fully incorporate chemical metabolism and therefore, may not fully account for chemicals metabolized to a more toxic form. This challenge calls for innovative thinkers to find new ways to incorporate physiological levels of chemical metabolism into high-throughput screening assays. The goal is to eventually retrofit existing assays with this new technology to evaluate both chemicals and their metabolite products.

This challenge has three stages; 1) Call for Proposals, 2) Prototype Development and 3) Product Testing. The first round is currently open and closes April 8, 2016. There are monetary prizes with each stage with a total prize pool of $1,000,000. Please distribute challenge information.

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High-throughput Screening Data Used in IARC Monograph

For the first time, high-throughput screening data was used in an International Agency for Research on Cancer (IARC) Monograph, which identifies and evaluates environmental causes of cancer in humans. ToxCast and Tox21 data were used to support the mechanistic evaluation of the potential impact of diazinon (an organophosphate insecticide) and its metabolite, diazoxon, on carcinogenesis. CSS scientist, Dr. Matt Martin of the National Center for Computational Toxicology (NCCT), was a member of the interdisciplinary working group that reviewed the data and drafted the monograph.

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CSS in the News

Recent News Articles

- **[Can Toxcast Be a Useful Tool in the Search for Obesogens?](#)**

- **[Come and Get It! More Data in iCSS ToxCast Dashboard v2](#)**

- **[Will Androgen be the New Estrogen?: Using High-Throughput Toxicity Data](#)**

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Meet CSS Scientists

- [Tina Bahadori](#)
- [Tom Knudsen](#)
- [Bill Mundy](#)
- [Sandy Raimondo](#)
- [Tim Shafer](#)
- [Dan Villeneuve](#)
- [John Wambaugh](#)

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