MIT CEHS NEWSLETTER
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First look at How Arsenic Alters Gut Microbiome and Metabolites

Article by Carol Potera, writer for Environmental Health Perspectives

This article is a result of the CEHS Pilot Project funding awarded to Dr. Kun Lu, along with Professors Steven Tannenbaum and James Fox. Dr. Lu is now in a faculty position at the University of Georgia.

The gut microbiome metabolizes the environmental toxin arsenic, generating several intermediate compounds like dimethylarsinic acid that are more or less toxic. The mechanisms behind these biotransformations and their consequences on human health are an ongoing area of research.

In turn, ingested inorganic arsenic changes the composition of gut bacteria. Researchers exploring this newer angle find that not only are members of bacterial families altered, but also metabolites made by gut bacteria differ between mice drinking arsenic-spiked water and controls. The results described in this issue of *EHP* suggest that arsenic-induced changes in the gut microbiome and related metabolites may affect human diseases. Arsenic exposure is linked to diabetes and cardiovascular disease and skin, bladder, lung, and liver cancers. "But there's a gap in our understanding of how arsenic influences the gut microbiome," says study leader Kun Lu at the University of Georgia, Athens.

To obtain an overview of how arsenic interacts with the gut microbiome, Lu's team used 16S rRNA gene sequencing to measure fluctuations of gut bacteria in C57BL/6 mice after they drank water spiked with 10 ppm arsenic for 4 weeks. Additionally, several hundred metabolites in urine, blood, and feces were analyzed with liquid chromatography/mass spectroscopy to obtain a global profile of how the bacterial changes impact metabolic function.

In control mice drinking arsenic-free water, the leading gut bacteria were *Bacteroides* (42%) and *Firmicutes* (53%). *Bacteroides* remained similar in arsenic-treated mice, but several *Firmicutes* families significantly decreased. *Firmicutes* are important gut bacteria that produce short chain fatty acids used as substrates for energy production. High levels of *Firmicutes* are linked to obesity. Their decline following arsenic treatment hints that the toxin may have anti-obesity actions.

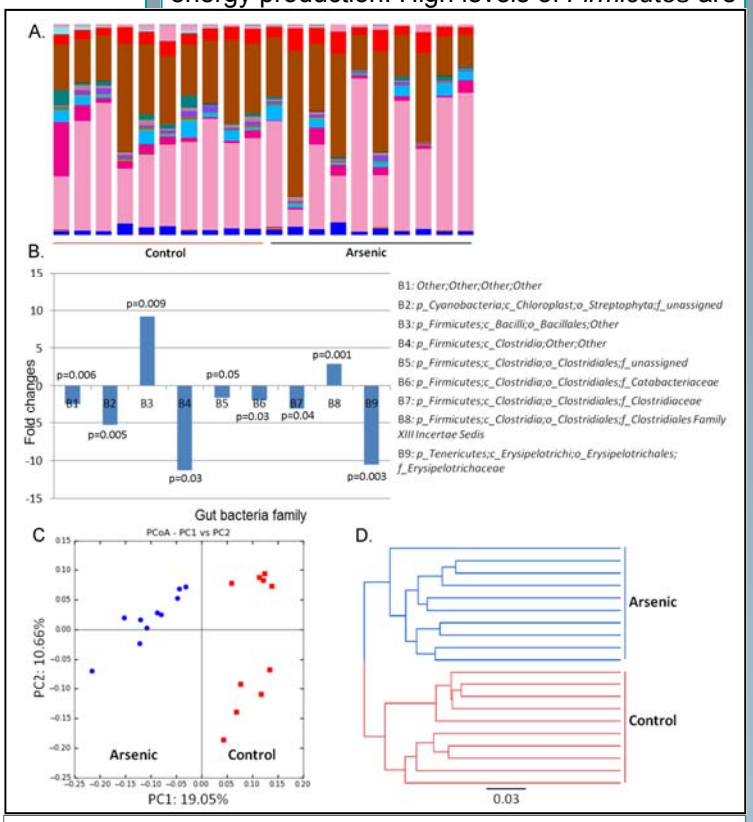

Figure 1. The gut microbiome composition profiles at *family* level in the control and arsenic-treated mice revealed by 16s rRNA sequencing (each color represents one bacterial family) (A); The fold changes and taxa assignments of significantly perturbed gut bacteria ($p < 0.05$) in arsenic-treated mice compared to controls (*p: phylum; c: class; o: order; f: family*) (B); The gut microbiome patterns of control samples (Red) and arsenic-treated (Blue) mice are readily differentiated by Principal Coordinate Analysis (C); Hierarchical Clustering analysis by the Unweighted Pair Group Method with Arithmetic Mean indicates that controls and arsenic-exposed mice cluster in their own groups, with the UPGMA distance tree constructed at distance of 0.03 (D).

The metabolic profiles showed that 146 metabolites increased and 224 decreased in feces following arsenic exposure, including bile acids, lipids, amino acids, and isoflavones. Some metabolites are linked to obesity, insulin resistance, and cardiovascular disease. For example, bile acids, which more than doubled

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In This Issue

Upcoming Events

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CEHS External Advisory Meeting to be held on March 4-5, 2014

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Robert S. Harris Lecture to be held on April 3, 2014. The speaker will be Professor Joseph Graziano (Columbia U.)

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CEHS Annual Poster Session to be held on May 9, 2014

CEE Team Looks at How Anthropogenic Emissions Interact with Organic Compounds Emitted by Trees

Article published by Denise Brehm of Civil & Environmental Engineering at MIT on July 3, 2013

Professor Jesse Kroll and members of his research group are doing fieldwork this summer at a site in the Talladega National Forest in Alabama as part of the National Science Foundation's Southeast Atmosphere Study (SAS), an umbrella study comprising five projects undertaken by scientists from 30 research institutions.

The goal of the SAS is to learn more about the processes that control biosphere-atmosphere interactions affecting regional climate and air quality in the southeastern United States, one of the few places in the world that has cooled during the last century.

At the field site in a clearing in the forest, Kroll, postdoctoral associate Eben Cross and graduate student Jon Franklin are looking at the chemical reactions between anthropogenic air pollution and the organic compounds emitted by trees.

Professor Jesse Kroll, Eben Cross, and Jon Franklin are participating in a large study of the atmosphere in the southeastern United States. They set up their equipment in one of the trailers at this field site in the Talladega National Forest in Alabama. *Photo: Southeast Atmosphere Study*

Organic compounds emitted into the atmosphere as gases react with other compounds to form particles of organic aerosol, which can continue to evolve through oxidation into secondary aerosols of large compounds. The dense deciduous trees in the Southeast emit organic compounds that form high concentrations of these secondary organic aerosols. Scientists think enhanced secondary organic aerosol formation may be responsible for the cooling effect.

But how emissions from anthropogenic activity, such as the nitric oxides in vehicle emissions, influence the amount and properties of this secondary organic aerosol is unknown. Any such effect likely would be particularly strong in the southeastern U.S., because of the substantial amount of anthropogenic activity and the very high emissions of biogenic volatile organic compounds from the forest.

"If nitric oxides or some other anthropogenic pollutant enhance biogenic secondary organic compound formation, that could explain the cooling trend in the Southeast," says Kroll. "We're going after this same topic in the laboratory, but the only way to really be sure we're realistically capturing the conditions of the atmosphere is to make measurements in the field."

To understand and be able to predict organic aerosol, scientists need to understand the life cycle of all organic species in the atmosphere — emissions, chemical transformations and deposition — in both the gas and particle phases. But instruments typically miss two important classes of organic compounds, intermediate-volatility and semi-volatile species, which are volatile enough to be in the gas phase, but "sticky" enough to attach to surfaces, says Kroll.

To capture these compounds in their measurements, Kroll and his team developed a new mass spectrometric instrument that can measure these nebulous species of low-volatility organic compounds. He says the key is to measure the compounds as an ensemble, as opposed to trying to quantify all individual species. They deployed their new mass spectrometer in one of the 12 research trailers located at the field site. "Having a real-time measurement of intermediate and semi-volatile gas phase organics in the atmosphere is somewhat uncharted territory in atmospheric chemistry, but with our instrument we think that we can obtain quantitative estimates of the amount and chemical characteristics of these important species," says Cross. "The regional summertime haze throughout the Southeastern U.S. may be a result of the combined influence of biogenic and anthropogenic emissions. Measuring intermediate and semi-volatile gas phase organics in a forested site downwind of urban areas will help us unravel the atmospheric chemistry in that haze."

Other projects in the Southeast Atmosphere Study involve two aircraft that are sampling air chemistry from the Mississippi River to the Atlantic Ocean, and from the Ohio River Valley to the Gulf of Mexico; ground instruments to measure low-level winds, moisture and temperature; and instrumented towers taking measurements within and above the forest canopy. The study is supported by the National Science Foundation, the U.S. Environmental Protection Agency and the U.S. National Oceanic and Atmospheric Administration.

Published on Department of Civil & Environmental Engineering, MIT (http://cee.mit.edu)

CEHS News

Welcome New Faculty Center Members

We are pleased to announce four new Center Faculty Members.

Steven R.H. Barrett, Assistant Professor of Aeronautics and Astronautics, who's research interest is in aviation and ground transportation climate and air quality impacts, alternative aviation fuels, geoengineering, low-emissions propulsion, and contaminant dispersion. Please visit his website for further information of his research and publications http://lae.mit.edu/?page_id=193

Jesse H. Kroll, Associate Professor of Civil & Environmental Engineering and Chemical Engineering, who's research involves the experimental study of the properties and chemical transformations of organic species in the Earth's atmosphere. Please visit his website for further information of his research and publications http://cee.mit.edu/kroll

Bradley L. Pentelute, Assistant Professor of Chemistry, who's research interest is in developing practical research tools for chemical biology, particularly engaged in designing new intracellular delivery platforms capable of transporting a wide range of molecular targets directly into the cytosol. Please visit his website for further information of his research and publications http://pentelute.weebly.com/

Noelle E. Selin, Assistant Professor of Engineering Systems Division and Earth, Atmospheric and Planetary Sciences, who's research uses atmospheric chemistry modeling to inform decision-making on air pollution, climate change and hazardous substances such as mercury and persistent organic pollutants (POPs). Please visit her website for further information on her research and publications http://web.mit.edu/selin/www/about_me.html

2013-2014 Pilot Project Awardees from Special Junior Investigator Call

The MIT CEHS allocates a significant portion of its NIEHS P30-ES002109 funding to support pilot projects that: provide initial support for investigators to establish new lines of research in environmental health, allow explorations of innovative new directions representing a significant departure from ongoing research for established investigators in environmental health sciences, and stimulate investigators from other fields to apply their expertise to environmental health research. The CEHS has awarded two translational pilot projects with a start date of December 1, 2013 as a result of a special pilot project call geared towards junior investigators.

The Translational Pilot Project award recipients and their project titles:

- **Jesse Kroll**, Associate Professor of Civil & Environmental Engineering and Chemical Engineering, and Eben Cross, Research Scientist of Civil & Environmental Engineering, "*Laboratory Characterization of the Composition and Variability of Solid Biofuel Combustion Emissions*".
- **Elizabeth Nolan**, Assistant Professor of Chemistry, "*Nickel Allergy and Proinflammatory Proteins".*

Pentelute receives Sontag Foundation Award

Announced October 4, 2013, **Professor Brad Pentelute** has been selected by The Sontag Foundation to receive a 2013 Distinguished Scientist Award. The award is given to outstanding early career scientists with inspiring, potential-laden brain cancer research proposals. http://chemistry.mit.edu/pentelute-receives-sontag-foundation-award

Focus on Instrumentation in the Imaging Facilities Core

The CEHS Imaging Facilities Core maintains a number of instruments for use by CEHS researchers. Among the available systems, the Cellomics ArrayScan (photo below) provides the most powerful and

flexible platform for automated quantitative image analysis. This instrument produces large datasets for multiparameter analysis of cell features. The Cellomics ArrayScan has a wide range of capabilities to quantify changes in whole cell and subcellular morphology. This system can use both brightfield images (transmitted light) and images produced by fluorescent -based reagents to capture high-resolution images of cells grown in a variety of formats. The CEHS ArrayScan is equipped with a **Live Module** for quantitative kinetic imaging of cells under conditions similar to those in a tissue culture incubator. A robotic plate handler is also available to facilitate large-scale experiments. Analysis of collected images is facilitated by software including BioApplication modules that are designed to quantitatively analyze targets within fixed or live cells. Some of the BioApplications available include the **Morphology Explorer** for the analysis of the shape or area of entire cells as well as their spacing and proximity or larger features such as clusters or assembly of cell colonies. It can also evaluate biological response by quantifying changes in subcellular features including the intracellular location, arrangement and structure of cellular organelles or the cytoskeleton. Translocations of signaling proteins among cellular compartments can be monitored using the **Cytoplasm to Cell Membrane Translocation** BioApplication. The **Target Activation** application provides measurements of intracellular fluorescence intensity on a cell-by-cell basis, performing much like a flow cytometer would analyze a cell population. Multiple parameters can be acquired and analyzed with the flexibility to identify organelles and specify the intracellular region where intensity measurements are made (e.g., nucleus or cytoplasm). Toxicity studies can be conducted with the **Multiparameter Cytotoxicity** application that is designed to quantify changes in nuclear size and morphology, membrane permeability and lysosomal functioning. The CEHS also has acquired BioApplications for **Molecular Translocation**, **G Protein-coupled Receptor Signaling** and **Tube Formation** (e.g., angiogenesis). Users can also develop their own applications with existing software.

CEHS members should also be aware of the Typhoon FLA 7000 Imager (photo below) which is a relatively

recent addition to the Imaging Core. Often referred to as phosphorimagers, the current versions of these instruments have additional capabilities with broader applications. The Typhoon is a versatile laser scanner with several imaging modes. It can perform quantitative analysis of radioisotopes using phosphor screens, chemifluorescent detection of Western blots with ECL reagents and gel documentation using visible stains (e.g., Coomassie blue). Phosphor screens are available for experiments using ^{32}P. The instrument is also equipped with an ImageQuant software package for quantitative analysis of acquired data.

Both instrumentations are available to Center members free of charge. The Cellomics ArrayScan is located in 16-306 and the Typhoon FLA 7000 Imager is located in 16-318. Please contact Dr. Bob Croy (rgcroy@mit.edu) for more information about capabilities and access to these instruments.

CEHS COE^{2}C HIGHLIGHTS

CEHS COE^{2}C Expands to New Audiences and Communities

By Kathleen Vandiver, CEHS COE^{2}C Director

Several new faculty members recently joined MIT's Center for Environmental Health Sciences (CEHS) bringing new fields of investigation and expertise. Thus the Community Outreach Education and Engagement Core (COE^{2}C) has expanded to new audiences to reflect the Center's widening research interests. Therefore, the COE^{2}C has recruited new partner organizations for working in these communities. For example, the Mystic River Watershed Association immediately provided us with several excellent networking opportunities in the twenty-two towns within the Aberjona and Mystic River watershed. The COE^{2}C has also broadened the participation of its Community Advisory Board (CAB), hosting thirty-two members at an advisory and community meeting on October 8, 2013 at Simmons Hall on the MIT campus.

Professor Harold Hemond is widely recognized for his historic investigative work during the 1980s on the contaminated Mystic River soil sediments which affected the drinking water supply in Woburn, MA. Now Professor Hemond is specifically interested in studying with CEHS what has happened to these river sediments thirty years later. This would involve nearby towns, such as Chelsea and Everett, situated near the mouth of the Mystic River. These towns have additional toxic burdens due to Boston's industrial port facilities. In recent months, these communities have been besieged by competing proposals for the construction of a major resort casino, which the Commonwealth of Massachusetts has just allowed. The COE^{2}C will be meeting with community groups to find out how we can be of assistance regarding the environmental health issues these proposals raise.

The addition of two new junior faculty members to CEHS from atmospheric and engineering disciplines has provided the COE^{2}C with the ability to focus on air pollution. Associate Professor Jesse Kroll's Lab conducts analytical studies on pollutants which develop secondarily from the ongoing chemical reactions occurring in the atmosphere. Assistant Professor Noelle Selin's Lab models the fate and transport of airborne hazardous substances on a global scale. She applies her knowledge to policy decision-making with other scientists on international panels. The COE^{2}C has become more invested in partnering with the American Lung Association (ALA) of the Northeast region. The COE^{2}C has facilitated briefing sessions between the ALA and MIT faculty on two different occasions this fall, where researchers discussed the potential health implications of their cutting edge research. The Community Advisory Board members at the October meeting were pleased to hear about these briefings, recommending that the COE^{2}C seek out opportunities and new ways for more researchers to advise decision makers.

COE^{2}C Community Advisory Board reception in the lobby of Simmons Hall at MIT in Oct. 2013

COE^{2}C Community Advisory Board members discussing ideas during roundtable discussions.

The Community Advisory Board members were also updated about our COE^{2}C programs for health professionals as well. On September 27, 2013, the COE^{2}C led a hands-on workshop for the faculty at Boston College's William F. Connell School of Nursing. This school is affiliated with the Harvard Catalyst, a program supported by the NIH Clinical and Translational Science Award (CTSA). This faculty workshop and our regular annual workshops for health professionals are direct efforts of MIT's Clinical Research Center Nurse Director, Dr. Catherine Ricciardi, DNP. She organized two nurse focus groups in which the COE^{2}C became highly aware of how local nurses struggle to gain sufficient fundamental molecular biology knowledge to keep up with modern medicine, such as understanding gene/environment interactions. Since the nursing profession is arguably the general public's most trusted profession, it seems evident that disseminating MIT's engaging instructional methodology could have broad translational impact. The COE^{2}C Community Advisory Board enthusiastically encouraged us to share our models nationally, and to present it at an all-day workshop at the American Public Health Association (APHA) conference in New Orleans, LA next year.

NIEHS Training Grant Highlights

The Training Grant in Environmental Toxicology (Professor John Essigmann, Principal Investigator) holds annual Responsible Conduct in Research sessions in the early part of each calendar year which all trainees are required to participate. The RCR sessions run for six weeks and each trainee participants take a turn at leading the discussion at their selected session. The 2014 Program include the following elements:

Week 1: Book Discussion: *The Immortal Life of Henrietta Lacks*, by Rebecca Skloot
Faculty Presenters: Linda Griffith and Bevin Engelward
Trainee Presenters: Bridget Wall and Nicole Billings

Week 2: The Lab," Part I – Interactive video program providing multiple pathways for avoiding scientific misconduct)
Faculty Presenter: Steven Tannenbaum
Trainee Presenters: Bridget Wall and Rebecca Lescarbeau

Week 3: The "Red Team III." From the series "The Newsroom" – Reporters, like scientists, collect, analyze and publish information. They also are tempted to exaggerate or downright fabricate data to make a better story. To minimize misconduct, they use the "Red Team" strategy to pressure test data and conclusions. The Newsroom is an Emmy Award winning HBO series that addressed this issue in its episodes dealing with data on a weapons of mass destruction story.
Faculty Presenter: Jacquin Niles
Trainee Presenter: Marcus Parrish

Week 4: Data Collection and Integrity, Intellectual Property – Students will excerpt fragments from "Glory Enough for All," a film that details the discovery and commercialization of insulin. Students also researched the use of electronic notebooks, and presented to the group the pros and cons of this new form of record keeping, as compared to pen and ink on paper.
Faculty Presenters: Ram Sasisekharan and Katharina Ribbeck
Previous Trainee Presenter: Charles Knutson

Week 5: "Race for the Double Helix" – Much of the pace and style of modern biological research was set in the 1950s, and the pressure we all feel to publish and have high impact is captured in this film. The film is rife with ethical issues that stimulate one to pause and think about how science is done and how we treat our colleagues. (This film was originally titled "Life Story" put out by the BBC). http://digital.films.com/play/UZSWS6
Faculty Presenters: Forest White and Douglas Lauffenburger
Trainee Presenters: Kim Davis

Week 6: "Cross-cultural Ethical Issues in Global Collaborations" – More and more companies rely on overseas partners to do research leading to drug discovery, to assure quality control and to do marketing. Glaxo Smith Kline, Sanofi and Novartis have been charged with unethical behavior stemming from their overseas operations. Trainees will be assigned the task of investigating these cases of alleged misconduct and reporting on what is being done, or should be done, to assure ethical behavior in the era of outsourcing.
Faculty Presenters: Jonathan Runstadler and Leona Samson
Trainee Presenters: Irene Blat and Nathan Stebbins

If any CEHS members are interested in attending he 2015 sessions, please contact Kim Bond Schaefer (kbond@mit.edu) who coordinates this important annual program.

Finally we would like to congratulate our two Trainees, Nicole Iverson and Bridget Wall, both supported on the Training Grant, who have won CEHS Poster Session awards this past May. The work of Dr. Iverson published in Nature Nanotechnology was also profiled on CNN News. Additionally, Marcus Parrish, who was appointed on the Training Grant took the 1st place poster award!

CEHS Featured Article Continued

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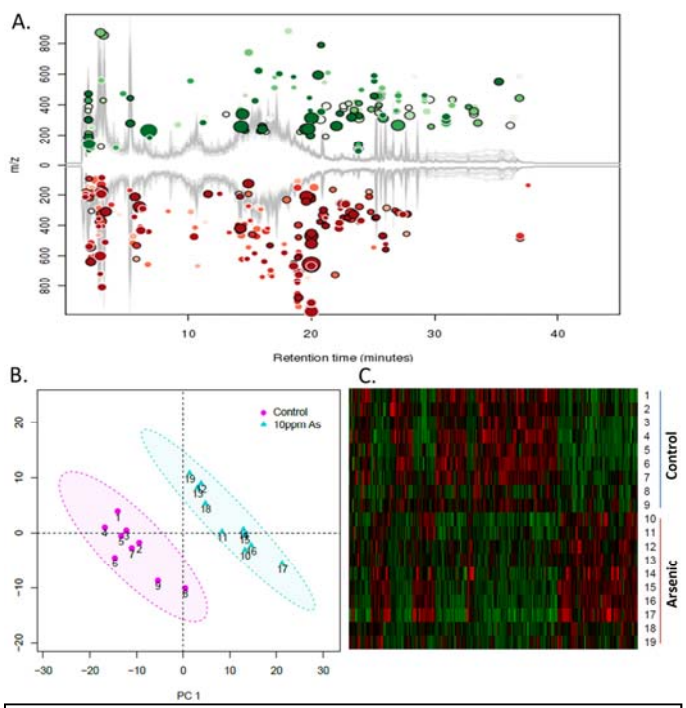

Figure 2. Arsenic exposure perturbed the metabolic profile of fecal samples of mice, with 370 molecular features being significantly changed compared to controls (fold change > 1.5 and p < 0.05) (Red: decreased metabolites; Green: increased metabolites) (A); Control animals are separated from arsenic-treated mice using metabolite profiles by Principal Component Analysis (B); The hierarchical clustering heat map constructed using molecular features with 1.5 fold changes (p < 0.05) shows a consistent clustering pattern within individual groups (C). (Control: sample 1 to 9; Arsenic-treated group: sample 10 to 19).

in arsenic-exposed mice, [Editors: This info comes from Table S2 (Supplemental Materials] aid the absorption of lipids and fat-soluble vitamins from the gut. Bile acids also act as signaling molecules in lipid metabolism and raise the risk for insulin resistance. "Bile acids may be potentially involved in arsenic-induced insulin resistance, but this needs to be confirmed," says Lu.

Overall, the integrated results provide preliminary mechanistic insights into how environmental toxins disrupt the gut microbiome and metabolites that induce human disease. In addition to arsenic, "we need to pay attention to the interactions of other environmental toxins like mercury that also are metabolized in the gut," says Lu.

"Lu's fascinating study uses state-of-the-art technologies to demonstrate that arsenic exposure modifies the gut microbiome. Given the likely probability that the effects they observed in the mouse could occur in the human gut as well, the findings have great importance for public health as millions of individuals are exposed to harmful levels of arsenic in their drinking water," says toxicologist Rebecca Fry at the University of North Carolina, Chapel Hill.

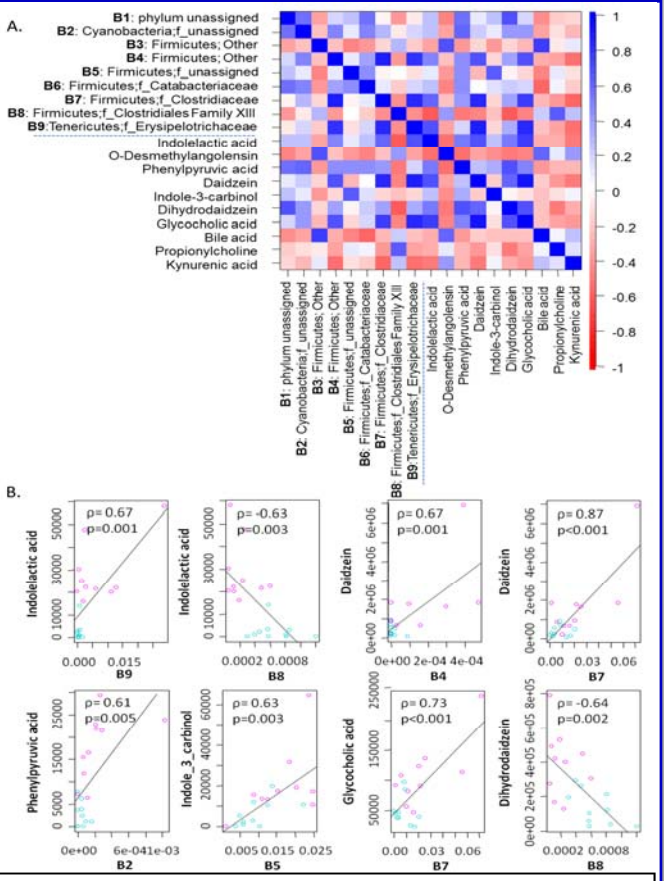

Figure 3. The correlation plot, calculated by Pearson's correlation coefficient, demonstrates the functional correlation between perturbed gut bacteria families and altered fecal metabolites (A); The scatter plots illustrate statistical association (ρ > 0.5 or < -0.5 and p < 0.05) between the relative abundance of altered gut bacteria families and the mass spectrum intensities of some typical gut-flora-related metabolites, including indole-containing compounds, isofavone metabolites and bile acids (B). (Pink: control samples; Blue: arsenic-treated mice.)

Worldwide, hundreds of millions of people drink water contaminated with inorganic arsenic levels that far exceed the 10 ppb guidelines of the US Environmental Protection Agency. In the United States, 25 million people drink water from private wells with arsenic levels above 10 ppb because the EPA does not regulate private wells.

A logical next step would be a human population study that compares gut microbiomes and metabolic profiles of people who drink well water naturally contaminated with high levels of inorganic arsenic to another group who drink tap water with arsenic below the EPA's cutoff. Many other questions, including dose-response and gender effects and persistence of gut microbiome changes, need to be addressed in future animal studies, says Lu.

The full publication of this article can be found here: http://ehp.niehs.nih.gov/121-a148/