RETURN REPORT: SABBATICAL LEAVE

Britton Ranson Olson**,** Biology

Sabbatical Leave: AY 2016-2017

Title of Sabbatical Leave Proposal: Investigating the Cellular Effects of Perflourinated Chemicals: the Development of a Research and Educational Cell Model.

Project Description

Background

My research interests are directed towards understanding the toxicity mechanisms of perfluoroalkyl substances (PFASs). Through the collaborative efforts of myself and researchers at Bowling Green State University, we have identified and developed a model for studying the cellular effects of PFAS exposure. The first part of my sabbatical was directed towards the development and refinement of a PFAS transport assay for the goal of understanding how these chemicals interact with the cells. The new method was successful, as I spent the fall developing the protocol and spring finalizing the data relevant to our research model. I presented the details of this methodology and those results at the American Society for Microbiology (ASM) national meeting 'Microbe' in June, 2017. The second part of this project was aimed at applying the CRISPR/Cas gene editing system to our toxicity model, along with making this valuable molecular system available to LSSU students. Over the period of the sabbatical my collaborator and I submitted three grant proposals seeking funding to generate the genomic sequences required to implement this system. Though more delayed than we had hoped (the first two submissions were not funded), we did ultimately receive those funds and now have the complete genomic sequences of ten previously undescribed strains in hand to implement CRISPR. The drafts will be used as the educational platform for the BIOL421 Advanced Cell and Molecular Biology course starting this spring, 2018, where students will learn about and participate in the design of the CRISPR/CAS system as applicable to our novel bacterial toxicity system. This will also provide many projects suitable for the student senior thesis.

As part of my leave I also prioritized identifying and networking with individuals within the scientific community regarding the topic of PFASs and this toxicity project. There are unique challenges that come with this project. First, this is a new cell model, and so proof of concept is required. Additionally, most existing PFAS research comes from Europe and Asia and is just gaining acknowledgement here in

the United States, meaning funding sources are difficult to identify. For instance, one of my sabbatical objectives was to create an RUI proposal, but I learned early on in the leave from program directors at the National Science Foundation (NSF) Integrated Organismal Systems program, that the NSF does not fund ecotoxicology-based studies with the rationale that they should be reviewed by the Environmental Protection Agency (EPA). I then found that the EPA did not have a publically available mechanism that aligned with these studies. I subsequently furthered my outreach to the Green Institute, EPA Great Lakes Restoration Initiative management, and the Sea Grant program, and with their feedback found the impetus for my project shifting towards the development of a broad toxicity model, one not specific for studying PFAS toxicity, but for assessing emerging contaminants in general, and one that could be used to analyze their effects on organisms that metabolize differently. One of the biggest achievements I made during this leave was a technical presentation that I was invited to give to senior scientists from the EPA and US Army and Engineer Research and Development Center in December, 2016. Their response regarding our novel model was favorable and they made the recommendation to further pursue its development and those steps necessary for its validation. We are currently following through on these recommendations of which two LSSU senior thesis projects have culminated. Students will gather this data and present it during the fall 2018 and spring 2019 semesters and we will continue to seek funding in this area.

In closure I would like to thank the sabbatical committee and the University for offering me this time and show of support for my research efforts. I look forward to bringing the tools and opportunities gathered here to the classroom and I hope that the students will be as inspired as I have been.

Sabbatical Outcomes

1. **Research Findings**

   CPC-ECR assay for PFASs. An anionic exchange reaction between 1mM cetylpyridinium chloride (CPC) and 1 mM eriochrome cyanine R (ECR). The net amount of PFAS measured through the absorbance emitted from the CPC-ECR complex at 626 nm, which is disrupted in the presence of PFAS. Our PFAS uptake measurements demonstrate that the PFASs have different profiles; while PFOS was detected within cell lysate fractions and that amount increased with increased incubation time, PFOA remained within the cell culture supernatant. These findings also suggest different modes of toxicity.

   Complete genome sequences of ten previously undrafted bacterial strains. Completion of a genome-phenome analysis will allow us to identify the PFAS cell targets with the potential to determine their toxicity mechanism(s).

   Growth curve analysis. While preparing a manuscript with the CPC-ECR data, it was discovered that a set of growth experiments performed previously by a post-doc at BGSU were done

incorrectly and bared repeating. I completed those experiments with the wildtype strain grown in aerobic-dark, anaerobic-light, and fermentation conditions.

Manuscript in preparation. Perfluorooctanoic acid and perfluoroctane sulfonic acid toxicity studies in the bacterial model *Rhodobacter sphaeroides* 2.4.1. Jill Zeilstra-Ryalls,[a] and Britton Ranson-Olson[b], [a] Bowling Green State University, [b] Lake Superior State University.

Student thesis projects. Renee Resendes 'PFAS Effects on the Development of Zebrafish' and Nicholas Hansen 'A Comparison of PFAS Effects on ATP Production in *Rhodobacter sphaeroides* and *Saccharomyces cerevisiae*'.

2. Research Presentations

Technical presentation December 19th, 2016. The potential of our model to serve as a broad model of toxicity reviewed by Gerald Ankley, U.S. EPA Environmental Effects Research Laboratory; Edward J Perkins, Senior Scientist (ST) Environmental networks and toxicology, US Army Engineer Research and Development Center; Dan Villeneuve, U.S. EPA Mid-Continent Ecology Division (MED).

ASM 'Microbe', June 1-4th, 2017. Investigation of the Effects of Perfluorinated chemicals using *Rhodobacter sphaeroides* as a Cell Model. Jill Zeilstra-Ryalls,[a] and Britton Ranson-Olson[b], [a] Bowling Green State University, [b] Lake Superior State University. New Orleans, LA.

3. Proposals

Department of Energy. Joint Genome Institute small grant. A genotype-phenotype study to identify cellular and molecular targets of perfluorinated carbon compounds. 2016. Co-PIs Britton Ranson-Olson and Jill Zeilstra-Ryalls. Award – draft sequence data. Not funded.

Environmental Protection Agency. Unsolicited mini-grant. Development and utility assessment of a broad, biologically based screening method for contaminants of emerging concern. 2017. Co-PIs Britton Ranson-Olson and Jill Zeilstra-Ryalls. Tabled pending data review.

Building Strength Grants Program, Bowling Green State University. Determining the genetic basis of perfluorinated carbon compound (PFC) toxicity. Co-PIs: Jill Zeilstra-Ryalls, BGSU and Britton Ranson-Olson LSSU. 2017. $10,000. Funded.

Fund for LSSU. Development of a Biologically Based Screening Method for Perfluorinated chemicals and Other Contaminants of Emerging Concern. 2017. $1,154. Funded.

Michigan Sea Grant. Core research proposal. A genome-phenome analysis to identify the cellular toxicity targets of perfluorinated chemicals. 2017. PI: Britton Ranson Olson. $100,000. Not funded.

The Agilent Seahorse XFp Analyzer Grant. Instrument grant. PI- Britton Ranson Olson. Pending review.

4. Technical communication/meetings

National Science Foundation. Kimberly Hammond and Micheal Mishkin, Program Directors, Physiological and Structural Systems Cluster, Integrative Organismal Systems Aug-Nov, 2016.

U.S. EPA. Santhini Ramasoma, National Center for Environmental Research, Nov 2016. Edwin (Ted) Smith, Chief, Database and Contracts Management, Great Lakes National Program Office Nov 2016- Jan 2017.

Green Science Policy Institute. Arlene Bloom, Director Nov 2016 and participated in the Flourinated Chemicals Meeting "Highly Fluorinated Chemicals: A Sticky Issue" in April 2017.

Sea Grant Program. Catherine Riseng, Research Program Manager, Michigan Sea Grant Dec 2016-June 2017.

5. Collaborations

Continued collaboration with Bowling Green State University and Dan Villeneuve of the U.S. EPA Mid-Continent Ecology Division (MED).

6. Other

Co-hosted along with Dr. Martha Hutchens the Fall Michigan branch of the American Society for Microbiology meeting on the LSSU campus, Oct 21st and 22nd, 2016, entitled 'Defense Against the Viral Arts'.

Organized a student recruiting event at Sault Area High school. LSSU alum Emily Estep and I presented on the LSSU biology program Dec 9th, 2016.

Michigan State University Early Assurance Program student interviews and submitted endorsements on behalf of LSSU pre-professional committee, March 2017.