An overarching goal of Project 2 is to determine how airborne PCBs affect the development of metabolic syndrome (MetS), a cluster of conditions that includes obesity, hypertension, dyslipidemia, and hyperglycemia, which increase the risk of heart disease, stroke, and type II diabetes. Project 2 will determine how airborne PCBs affect the development of MetS in adolescence, specifically through their effects on adipose tissue. We will be doing this by:

**1) Elucidating the Functional Consequences Of Airborne PCB Exposure on Adipogenesis and Adipocyte Function**

For aim 1, starting in 2020 we have begun screening of lower chlorinated airborne PCBs and their metabolites to determine how these factors affect adipogenesis and adipocyte function. We have developed and implemented methods to assess lipid accumulation and alterations in secreted factors after exposure to PCBs. We have modified and streamlined the conditions for generating and culturing adipose organoids to make it easier to assess multiple concentrations and replicates. We first identified the size of adipose organoids that generate the most adiponectin. We found
adipose organoids made with just 5,000 pre-adipocytes generated the highest level of adiponectin per cell. The reduced size allows us to test more PCB congeners at once and the higher adiponectin output increases the sensitivity of our assay to detect a decline in adiponectin output. The higher adiponectin output has also allowed us to perform our screening assay in a 96-well format with 1 spheroid per well. This is a major reduction compared to the 35 spheroids/well format we had initially planned and further improves our ability to screen more PCB congeners and doses with a high number of experimental replicates. Each condition is replicated in 6 wells for each run of the experiment. The reduced demands on initial cell input will be critical as we move forward with studies using primary human preadipocytes. To further improve the rigor of our screening assays, we have developed SOPs and workflows that utilize robotic pipettors to streamline the handling and collection of samples. The progress we have made on this aim provides a strong platform for the next phase in our analysis, namely, to test the effects of the PCB congeners and mixtures on preadipocytes derived from different fat depots, donors, and sexes. The Data Management and Analytics Core will be closely involved with the analysis of the data generated from these studies.

In addition, to the above studies, we have made progress in determining how PCB52, an airborne PCB that is at high levels in school air and accumulates readily in serum and fat, causes biological changes to preadipocytes. Using RNAseq, we found that PCB52 and its common hydroxylated metabolite PBC52-OH cause alterations in gene expression patterns in preadipocytes are that have been associated with obesity and diabetes. Namely, for PCB52, changes were found in a pathway involving PPAR, a family of nuclear receptors that are master regulators of gene expression. Interestingly, the PCB52-OH metabolite causes changes that are different than its parent compound. Namely, changes in beta-catenin, inflammatory, cholesterol metabolism, microtubule dynamics, and other pathways were observed. These results demonstrate that metabolism of congeners such as PCB52 can result in broad changes in biological activity and strongly support the premise of aim 2 to develop a liver/adipose co-culture model that provides a system to achieve in situ metabolism of PCBs in the presence of adipose tissue. We will be working with the Data Management and Analytics Core to process the data related to these studies and to deposit it with public databases.

2) Developing a human adipose-liver biomimetic on-chip that allows for facile and accurate testing of the effects of environmental toxicants on adipocyte function

For specific aim 2, we have initiated studies to determine the best platform for culturing liver organoids such that they can be utilized in experiments to determine how lower-chlorinated airborne PCBs are metabolized. These can then be co-cultured with adipose organoids to assess how the metabolized PCBs affect adipogenesis and adipocyte function. For these studies, we are comparing available human liver cell lines in 2D and 3D cultures to determine which culture conditions provide for the best and most relevant metabolism. We will assess how the culture conditions compare with regard to long-term viability, expression of p450 enzymes, and metabolism of the common airborne PCB congeners and mixtures. The latter analysis will be performed in conjunction with the Analytics Core led by Dr. Lehmler.
3) Determining how airborne PCBs affect adiposity and metabolism

With Dr. Thorne from Project 3 and Dr. Lehmler from Project 1 we are in the process of planning inhalation and implant experiments in rats to deliver the airborne PCB congeners and metabolites to rats. We will determine how exposure to these compounds affect adipose tissue accumulation, adipose tissue inflammation, adipokine levels, and the metabolomic profile of the rats. These in vivo experiments have been significantly delayed because of the COVID-19 pandemic.

Aloysius Klingelhutz, Ph.D., Project Leader

Dr. Klingelhutz is a Professor of Microbiology and Immunology with expertise in preadipocyte immortalization and preadipocyte/adipocyte cell culture, 3D organoid culture, and assessment of parameters related to adipocyte function. He will be responsible for overall direction and management of Project 2, and will work closely with Ankrum to coordinate different aspects of this study, organize weekly meetings, and to supervise budget, data analysis, and dissemination of experimental findings through presentations, manuscripts, and progress reports. As Project Leader, he will serve on the ISRP Executive Committee and ensure all Project 2 activities are well integrated with the ISRP and supportive of the specific aims of all components.
James Ankrum, Ph.D., Co-Investigator

Dr. Ankrum is an Associate Professor of Bioengineering with expertise in bioengineering of cells, mesenchymal stem cells, and 3D organoid cultures. He will share leadership responsibilities with Klingelhutz and oversee the tissue-on-chip experiments. In addition to supervising and participating in daily activities in his laboratory relevant to Project 2, he will work closely with Klingelhutz to coordinate the overall project, manage experiments, analyze data, and disseminate experimental findings through presentations, manuscripts, and progress reports.

Larry Robertson, PhD, Other Significant Contributor

Dr. Robertson will work with Dr. Klingelhutz to design and interpret the rat studies as well as to provide guidance on in vitro PCB exposure studies. Dr. Robertson was a Project Leader for previous ISRP studies and was Director of the Iowa Superfund Research Program for 12 years.

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