Airborne PCBs and their Metabolites: Risk Factors for Adverse Neurodevelopmental Outcomes in Adolescence

Project 1’s long-term goal is to characterize the contribution of airborne PCBs and their metabolites to PCB neurotoxicity following exposure to airborne PCB congeners detected in indoor air of U.S. schools and, as a consequence, in school children and adolescents. The objective of this project is to inform future risk assessment by defining the link between neurotoxic PCB metabolites present in the brain and neurotoxic outcomes following adolescent exposure. We will accomplish our objective by:

1) Identifying cellular sites and targets of airborne PCB metabolites vs. parent compounds responsible for neurotoxicity in vitro

During 2020 as part of Aim 1, we performed initial studies investigating cellular sites and targets of PCB-induced neurotoxicity using C6 cells (rat glioma cell line) and primary glial cells (isolated from C57BL/6 mice). Cells were exposed to varying concentrations of PCB52 or two of its metabolites found in humans, namely, 4-OH-PCB52 or 4-PCB52 sulfate, for 24 hours. The C6 cells were similarly treated with three other PCB sulfates, namely, PCB3 sulfate, PCB11 sulfate, and PCB25 sulfate. Cell viability was determined by MTT assay. Results indicate that, in C6 cells and primary glia, 4-OH-PCB52 was the most cytotoxic compound (LC\(_{50}\) of 2.2 μM in C6 cells), followed by the parent compound, PCB52 (LC\(_{50}\) of 8.8 μM in C6 cells). The PCB52 sulfate was cytotoxic to C6 cells (LC\(_{50}\) of 8.4 μM) but not primary glia. These observations demonstrate the importance of the structure-activity relationship in PCBs mediated neurotoxicity. In addition, these findings suggest that astroglial cells may be a sensitive target for PCB52 and metabolites.

2) Characterizing the region-specific biotransformation of PCBs and PCB metabolites with in vitro models and in the adolescent rat brain in vivo

We have demonstrated that PCB metabolites are formed in the liver and distributed to the rodent brain, where they undergo further metabolism to neurotoxic metabolites. Building on these findings, we will test the hypothesis that metabolism of PCBs in neurons and/or astrocytes results in the local formation of neurotoxic metabolites of airborne PCBs.

3) Determining the effects of human metabolites of airborne PCB on biochemical markers of PCB neurotoxicity and behavioral outcomes in rats exposed throughout adolescence in vivo

In preparation for the animal studies proposed in Specific Aim 3, in 2020 we established a protocol to prepare the polymeric implants. Biphenyl-4-ol was used as an inexpensive model compound. Briefly, the core of the polymeric implants was prepared by dissolving 0.2 g polymer and 0.05 g F127 surfactant in 10 mL dichloromethane. After the solvent has evaporated, the pliable residue was extruded into silicone tubing and allowed to set for 24 h. The polymer was removed from the silicone tube and allowed to harden for another 24 h. Subsequently, this core is coated by repeatedly dipping it into a solution of 2 g P-80 with or without biphenyl-4-ol (5% by weight) in 20 mL of dichloromethane. These polymeric implants were dried for approximately 16 h to allow residual dichloromethane to evaporate.
cut into 2 cm segments, and stored at -20 °C. In vitro studies are underway to characterize the sustained release of biphenyl-4-ol from the implants. The same approach will be used to prepare polymeric implants for sub-acute neurotoxicity studies with 4-OH-PCB52, a human-relevant metabolite of PCB52.

**Project Leader: Hans-Joachim Lehmler**

Dr. Lehmler is a Professor in the Department of Occupational and Environmental Health at the University of Iowa (UI). He earned his PhD in synthetic organic chemistry from the University of Bonn, Germany, and received training in chemical and analytical toxicology at the University of Kentucky and the UI. He has extensive experience with assessment of the toxicity of environmental contaminants and their metabolites in vitro and in vivo. He serves as Director of the Environmental Health Sciences Research Center (EHSRC) and as Leader of the Synthesis Core of the Iowa Superfund Research Program (ISRP) since 2006. Together with Jonathan Doorn, he directs the Oxidative Stress and Metabolism Thematic Area of the EHSRC.

**Jonathan Doorn, PhD, Co-Investigator**

Dr. Doorn is an Associate Professor in the Division of Medicinal and Natural Products Chemistry, College of Pharmacy at the UI. He earned a PhD in Toxicology from the University of Michigan and received postdoctoral training in pharmacology/toxicology at the University of Colorado Health Sciences Center. He has much experience studying the role of neurotransmitter (dopamine) metabolism in neurotoxicity and disease and the involvement of pesticides.

**Michael W. Duffel, PhD, Co-Investigator**

Dr. Duffel is a Professor in the Division of Medicinal and Natural Products Chemistry, College of Pharmacy at the UI. He has extensive expertise in the enzymology of sulfation and sulfotransferases and in the oxidation and subsequent further metabolism of lower-chlorinated PCBs. This includes experience with a range of approaches from in vitro biochemical methods to quantitative structure activity relationships and studies with in vivo models. He will be directly involved in experimental design and interpretation of those aspects of Aims 1 and 2 relevant to metabolism of PCBs and interconversion of those metabolites.