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Overview:

Polycyclic aromatic hydrocarbons (PAHs) are toxic pollutants generated from the incomplete combustion of organic materials. They are thermostable, bioaccumulate in the environment and are known for their carcinogenic and proinflammatory properties.1-2 There are various routes of exposures to humans such as via ingestion, inhalation, and dermal contact.3 Several studies hypothesize that PAHs can influence our gut microbiome and their ability to perform their regular metabolic functions.4 Ultimately, exposure to PAHs may create a functionally dysbiotic gut microbiome that promotes gastrointestinal disease states.5

For this case study, we aliquoted 50mg of small intestine gut contents of male and female mice that had been exposed to a PAH mixture of benzo[a]pyrene (BaP), naphthalene and phenanthrene chronically and compared that to a control group of male and female mice that were not exposed to this mixture. In particular, the US Environmental Protection Agency (EPA) has recognized benzo[a]pyrene as extremely carcinogenic.6 The samples were processed similarly following a simple sample preparation protocol and a BCA assay (Bicinchoninic acid assay) was performed to allow for total protein quantification of these samples’ subsequently. The quantification of protein is a vital step when it comes to the measurement of enzyme activities as this would allow the normalization of the total protein concentration to the same amount across all experimental groups and would prevent biases when running enzymatic activities studies.

We adapted R code available online to process raw BCA assay data readings, taken on a plate reader that uses a colorimetric based analysis to quantify proteins. We plotted a graph of total protein concentrations against absorbances obtained. The code was able to find the equation specific to the least-squares regression line of our raw data. Using this code, we were able to determine the unknown protein concentration of gut content samples. In order to improve the plot and to display the names of the protein samples in the plot, we used ChatGPT to provide us with the code for performing BCA assay analysis using R. We compared the code that ChatGPT returned with the working code that we have. We also compared the plots that were obtained with and without the use of Chatgpt.

Our case study will give way for more downstream studies as quantifying total protein concentrations is vital for assessing the enzyme dysfunction that PAHs are hypothesized to cause in the gut microbiome.  The code that was generated will facilitate analysis using kinetic methylumbelliferone (MUB) assays, proteomics and active based protein profiling.