The Effects of Humic Substances on Acute Toxicity of Polycyclic Aromatic Hydrocarbons

*Baker, D.[1] , McAmis, B.[2], and Raychoudhury, S.[3]

[1]Undergraduate Research Assistant, Department of Biology, Chemistry and Environmental Health Science, [2]Research Associate, Department of Biology, Chemistry and Environmental Health Science, [3] Professor of Biology and Dean, School of STEM, Benedict College

Dajana.Baker45@my.benedict.edu

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Abstract: Polycyclic aromatic hydrocarbons are environmental pollutants, formed as byproducts of combustion and industrial processes. Their environmental prevalence poses major concerns and risks due to bioaccumulation and carcinogenesis potential. Humic acid (HA) is the byproduct of plant degradation, capable of PAH detoxification and degradation. The objective of this experiment is to measure the metabolic activity, viability, cytotoxicity, and apoptosis in MDA-MB-231 cells following their exposure to various PAH mixtures alone and together with HA. In this experiment one million cells were added to each 35mm dishes for 24 hours and on next day the cells from each dish were exposed to medium containing 1.16% acetonitrile (vehicle control), or in the presence of 10µM HA, 7µM PAHs alone and with 10µM HA. After 24 hour of exposure, the cells were trypsinized and collected in plastic tubes. Then we washed the cells in PBS. The pellets were then resuspended in propidium iodide (PI) solution for cell cycle analysis by flow cytometry. Flow cytometric analysis indicated all samples entered G1 phase at relatively equal amounts. However, the presence of PAHs by itself resulted in a sharp decrease of cells that entered S phase, indicating the highest level of cytotoxicity in PAH exposures. Cells exposed to the mixture of PAH and HA displayed reduced S phase DNA at the same level as it was found with PAHs alone. Through additional experiments utilizing multiple cell-lines and concentrations of HA, we are further investigating the role of HA in reducing bioavailability of PAHs in living cells.