Bioavailability and Toxicology of PAHs Associated With Soils and Sediments

Polycyclic aromatic hydrocarbons (PAHs) are a heterogeneous group of environmental contaminants. These compounds originate from petroleum products such as gasoline, diesel and jet fuels, and also can be produced by natural processes such as burning of organic material. The toxic effects of PAHs have received a great deal of interest, as the group includes compounds which are complete carcinogens and others which may serve as endocrine disrupters.

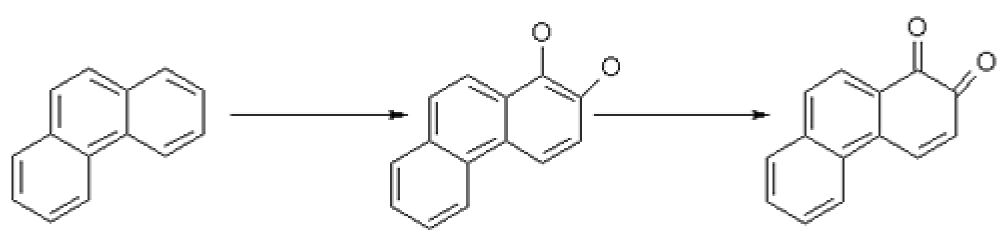
Interaction of PAHs with organic components of the soil matrix can modulate their interactions in the environment. Sorption/desorption experiments with soil humic material have shown that model PAHs can become sequestered—possibly into microporous domains in the organic matrix of the soil. If such sequestration limits access to bacteria which can degrade PAHs, then this association may lead to increased persistence of PAH contamination in the environment. Sequestration of PAHs, however, may also render them unavailable to higher organisms, potentially reducing their toxic effect.

Hypothesis

This research is based on the hypothesis that sequestration of PAHs by soil components such as humic acids reduces their bioavailability, and thus influences both their potential toxicity and their environmental persistence.

Research Plan

In order to investigate the bioavailability of sequestered PAHs, we are taking a multifaceted approach. Biological systems have the ability to rapidly transform PAHs into several different reactive intermediates (see Figure 1). Many of these PAH metabolites possess the ability to form adducts with biological macromolecules, such as DNA or proteins. Therefore, our first task is to develop analytical tools to identify these metabolites. The second part of the research plan is to determine the suite of metabolites produced by an individual organism, using subcellular fractions. Lastly, we shall conduct whole organism exposure studies using model sequestered PAH systems.



Phenanthrene **1, 2-phenanthrenequinone**

Figure 1: Two steps in the metabolism of phenanthrene, a polycyclic aromatic hydrocarbon. The first step is accomplished by CYP450 detoxifying enzymes, the second, by dihydrodiol dehydrogenase. The quinone form is a reactive metabolite, believed to be capable of adducting to both DNA and proteins.

Methodology

The first focus of the research plan is to develop analytical tools to detect PAH metabolites and to determine from them an estimate of total PAH exposure. To accomplish this goal, we are currently using TMAH-thermochemolysis GC-MS, in conjunction with other analytical tools, to study the formation of PAH metabolite adducts in small-molecule model systems. Following the refinement of these techniques, we shall apply them to cell-free enzymatic systems to establish metabolic profiles for a number of model PAHs. These studies will incorporate enzymatic systems from multiple test organisms, to determine differences in metabolite profiles due to differing enzymatic expression and activity. These studies will make use of the wide range of expertise available on our team, combining the analytical tools developed from small-molecule model systems with established biochemical techniques such as 2D-Polyacrylamide gel electrophoresis (2D-PAGE).

Sequestration of PAHs has the potential not only to influence the toxic exposure and response of higher organisms but also to alter the availability of these contaminants to PAH-degrading bacteria. Research in this area will focus on the ability of bacterial strains to eliminate model PAHs bound to different matrices. Additionally, the use of materials with defined micropore structure, such as zeolites, will allow us to investigate the effect of pore size and structure on bacterial bioavailability. Development of the analytical tools necessary to detect metabolites in eukaryotic systems will also be used here to investigate bacterial metabolites. The combination of approaches will serve to give us a more complete perspective on biological degradation of PAH contaminants in natural environments.

In conjunction with Dr. Yvonne Dragan from the National Center for Toxicology Research, we will be conducting whole organism exposure studies, to determine the availability of sequestered PAHs. This research will focus on two major exposure routes, both through contact with contaminated soils and through consumption. A secondary goal of this research is to determine the biological response of these organisms to exposure. The tools developed for the identification and quantification of PAH metabolites can also be used to assist in probing the toxic effect of these contaminants at a molecular level. These studies will be combined with recently developed microarray techniques, which will allow us to determine the toxic response of the organism at the RNA and protein expression levels.