

Abstract: Human CYP2A and respiratory tract xenobiotic toxicity

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The research goal of this revision proposal is related to *Reducing Toxicity of Tobacco Products and Smoke*, one of the four areas relevant to the *Family Smoking Prevention and Tobacco Control Act*. Specifically, we seek to gain a better understanding of the mechanisms underlying cigarette smoke (CS)-induced lung tumorigenesis in mouse models, in order to improve the utility of the mouse models for testing the carcinogenicity of tobacco products. We will address critical questions regarding the mode of action of the CS toxicants, the specific carcinogens involved, and the need for target-tissue bioactivation. The anticipated outcome will increase confidence in the predictive value of the animal model for risks of lung carcinogenesis in humans. We will also test a novel approach to increasing the sensitivity to CS-induced lung tumorigenesis in mice; the increase in sensitivity would make it possible to detect CS-induced lung carcinogenesis (as well as other toxicities, such as cardiotoxicity, acute respiratory toxicity, and developmental/reproductive toxicity) at lower doses of inhalation tobacco exposure. Success of our research will yield improved mouse models (with improved sensitivity and human relevance) that can be used to better compare overall toxicity between two different tobacco products (including those with reduced levels of harmful and potentially harmful constituents) in vivo.

The studies proposed for the revision project represent an expansion in scope of the parent grant, to including studies of CS-induced lung DNA damage and tumorigenesis. The revision project will also utilize transgenic mouse models, as well as expertise on carcinogen metabolism, DNA damage, and lung tumorigenesis, established in the parent grant.

The long-term objective of the parent grant is to determine the role of respiratory tract cytochrome P450 (P450 or CYP) enzymes in the metabolic activation and toxicity of environmental chemicals, with a focus on CYP2A13, a human enzyme selectively expressed in the respiratory tract, and on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a major tobacco-derived lung carcinogen. Our central hypothesis is that CYP2A13 plays a vital role in tobacco-related lung carcinogenesis.

In Aim 1 of the parent grant, we are determining the ability of specific mouse and human lung P450s to mediate NNK-induced DNA damage and lung tumorigenesis in mouse models. In the revision project, we will study lung tumorigenesis induced by CS, rather than by a single tobacco carcinogen, and we will study the combined activities of all microsomal P450 enzymes, rather than individual P450 enzymes.

Aims 2 and 3 of the parent grant, which are concerned with regulation of CYP2A13 gene expression and genetic polymorphisms of human CYP2A13, are not directly related to the revision project.

The proposed revision will not change the specific aims, research design, and methods of the current grant, but the anticipated results may influence future directions of the parent grant. Our studies to date in Aim 1 of the parent grant have shown that lung P450s play a major role in NNK-induced lung tumorigenesis in mice, and that human CYP2A13 can mediate NNK-induced lung tumorigenesis in a CYP2A13-humanized mouse model. A logical next step is to determine the role of CYP2A13 (and other individual lung P450 enzymes) in the lung tumorigenesis induced by not just individual carcinogens, but the actual tobacco smoke, as reviewers of our original proposal pointed out. These latter studies, which were not proposed in the parent grant, are premature at this time, given our poor mechanistic

understanding of the CS-induced lung tumor bioassays. Our plan is to first take a broad stroke, asking whether the CS-induced lung tumorigenesis in the mouse model actually requires P450-mediated bioactivation of any CS carcinogens (at levels and composition present in CS), and learning about the specific CS carcinogens involved (e.g., is NNK implicated?), as proposed in this revision project. The outcome will provide the basis for future testing of the specific roles of individual human P450 enzymes (e.g., CYP2A13) in CS-induced lung tumorigenesis in a mouse model. Success of these future studies will provide the rationale for incorporating human lung P450s or other biotransformation enzymes capable of bioactivating tobacco carcinogens into mouse models for testing carcinogenicity of various tobacco products. The incorporation of human lung P450s will also make it possible to test in vivo efficacy of chemopreventive agents that target human lung P450s for inhibition.