

## Determination of the Precursors to Polycyclic Aromatic Hydrocarbons in Mainstream Smoke by Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry

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**Hypothesis:** Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) can be used to determine the precursors to polycyclic aromatic hydrocarbons (PAHs) found in mainstream cigarette smoke (MSS) by spiked cigarettes with  $^{13}\text{C}$ -labeled PAH precursors. These studies could provide a bridge to relate pyrolysis and combustion data from tobacco model compounds directly to the fate of these components in a burning cigarette.

**Results:** The two major challenges in this proof-of-principle project were (a) developing an analytical method to cleanly separate the PAHs found in the TPM of 2R4F reference cigarettes for GC-C-IRMS analysis (i.e., obtain baseline resolution of the PAHs), and (b) determining if the sensitivity of the GC-C-IRMS was adequate to accurately measure small changes in the  $^{13}\text{C}/^{12}\text{C}$  ratio of the PAHs found in low (ng) levels in the TPM. Overall, this project was successful. A multi-step separation method was developed to isolate the PAHs found in MSS from 2R4F cigarettes, and the  $^{13}\text{C}$  content of phenanthrene, benz[a]anthracene, chrysene, and benzo[a]pyrene was measured by GC-C-IRMS. Unfortunately, the  $^{13}\text{C}$  content of the chrysene and benzo[a]pyrene could not be reproducibly determined because of poor chromatography and low concentrations, but lessons learned from this study provide a path forward to solve these issues. It was determined that GC-C-IRMS can reproducibly ( $\pm 3\%$  or  $1\%$ ) measure the  $^{13}\text{C}$  content of the PAHs found in the TPM at concentrations as low as  $10 \text{ ng}/\mu\text{L}$  ( $0.06 \text{ nmol}$ ), as long as good chromatography was obtained. By spiking 2R4F cigarettes with low levels ( $0.026 \text{ wt}\%$  or  $0.0052 \text{ wt}\%$ ) of cholesteryl stearate- $3,4\text{-}^{13}\text{C}_2$ , which served as a model for all steroids in tobacco, it was determined that steroids produced only  $2.6 \pm 0.5 \text{ wt}\%$  of the phenanthrene and  $2.5 \pm 0.5 \text{ wt}\%$  of the benz[a]anthracene found in the TPM, indicating that other tobacco components were responsible for producing the majority of these PAHs. The next step is to determine if cellulose is the dominant precursor of the benzo[a]pyrene found in MSS by spiking cigarettes with glucose- $^{13}\text{C}_6$  and analyzing the PAHs by GC-C-IRMS. If these experiments are successful,  $^{13}\text{C}$ -labeled cellulose will be synthesized, mixed with cigarettes, and the PAHs in the TPM will be analyzed by GC-C-IRMS.

**Conclusions:** For the first time, a quantitative relationship between tobacco precursor and mainstream smoke product can be obtained by a combination of  $^{13}\text{C}$ -labeling of tobacco precursors and GC-C-IRMS. This technique can also be used to gain insight into the mechanisms of PAH formation in a cigarette by determining the  $^{13}\text{C}$ -content of the reaction intermediates. GC-C-IRMS is also applicable to  $^{15}\text{N}$ -labeled tobacco components so the origin of nitrogen-containing polycyclic aromatic compounds could be investigated. Overall, GC-C-IRMS coupled with isotopically labeled tobacco components is a powerful and sensitive tool that can provide quantitative and qualitative information on product formation in a burning cigarette that cannot be obtained by other methods. The information gained from these experiments can be used to reduce the harm of cigarette smoke.

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