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Smoking Increases Carcinogenic Polycyclic Aromatic Hydrocarbons in Human Lung Tissue[1]

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Abstract

Tobacco smoke is a major source of human exposure to polycyclic aromatic hydrocarbons (PAHs). The concentration of PAHs in lung tissue would reflect an individual's dose, and its variation could perhaps reflect cancer risk. Eleven PAHs were measured in 70 lung tissue samples from cancer-free autopsy donors by gas chromatography-mass spectrometry. There were 37 smokers and 33 nonsmokers as estimated by serum cotinine concentration. The sum of PAH concentrations was higher in smokers ($P = 0.01$), and there was a dose-response relationship for greater smoking ($P < 0.01$). Smoking increased the concentration of five PAHs including benzo(*a*)pyrene, which increased ~2-fold. The risk for increasing carcinogenic PAHs (odds ratio, 8.20; 95% confidence interval, 2.39–28.09) was 3-fold compared with noncarcinogenic PAHs (odds ratio, 2.61; 95% confidence interval, 0.75–9.12). A higher concentration of PAHs was detected in the lung tissue of males, although the estimated smoking was similar in males and females. Race was not associated with PAH concentrations overall, but PAH concentrations appeared to be higher in African-American males than in any other group. Age was weakly correlated with an increase in fluoranthene and pyrene. The measurement of PAHs in human lung tissue can be used to estimate the actual dose to the target organ.

Introduction

BAP[3] and other PAHs are ubiquitous genotoxins present in cigarette smoke, air, water, and food. Seventeen PAH compounds, including BAP, have been classified as laboratory animal carcinogens (1). Strong epidemiological (2) and laboratory (3) evidence, including site-specific hotspot mutations in the *p53* tumor suppressor gene (4, 5), links these compounds to human lung cancer. PAHs are also implicated in other cancers including skin, head and neck, bladder, and colon (2).

The carcinogenicity of PAH compounds is mediated by DNA damage (3). The best-characterized pathway for genotoxicity of PAHs consists of covalent binding of the metabolically activated carcinogens to DNA bases to form DNA adducts (6). The DNA adducts are promutagenic lesions that can lead to mutations in oncogenes or tumor suppressor genes, if not repaired or otherwise eliminated (7). Concentration of DNA adducts is considered the biologically effective dose of the parent PAH (8). Because accumulation of parent PAH compounds in the lung tissue precedes formation of DNA adducts, it is intuitive that the lipophilic parent PAHs would be a good estimate of dose in the target organ.

Smoking yields of PAH compounds vary considerably based on the type of cigarette and human differences in smoking behavior. It was estimated by the FTC machine method that a smoker is exposed to about 1–30 μg/day/pack of cigarettes (9). PAH exposure also occurs from non-tobacco sources and can be assessed by environmental sampling or personal monitoring (10). Certain occupational exposures, *e.g.*, coke-oven work, present a burden comparable with smoking. The air in urban areas can contain higher percentage of PAH compounds than the air in the countryside, especially in the winter. Our diet also contributes to exposure, especially from overcooked foods; it has been estimated that diet contributes 3 μg/day of total PAH intake, ~70% for a nonsmoker (11). The PAH exposure pattern is therefore complex, and analysis of the lung tissue can provide direct information difficult to estimate by other methods.

Our study determined the concentration of 11 PAHs in 70 lung samples of cancer-free subjects using a sensitive and robust GC-MS method (12). Six of the analyzed PAHs were classified previously as laboratory animal carcinogens (1). The study reports the analysis of individual PAHs as well as the sum of carcinogenic, noncarcinogenic, and total PAHs. The size (70 individuals) and scope (11 PAHs) of the study provide an evaluation of the effect of smoking, race, gender, age, and fat content on the accumulation of PAHs in the lung tissue.

Materials and Methods

Population Description. The population consisted of 70 cancer-free Caucasian and African-American autopsy donors (32 males and 38 females; 31 Caucasians, 36 African-Americans, and 3 Hispanics; 37 smokers and 33 nonsmokers). The mean age was 35 years (range, 15–65). The tissues were collected through a medical examiner's office. Gender, race, age, and cause of death were determined by the medical examiner. The subjects died suddenly from multiple injuries in motor vehicle accidents ($n = 23$), gunshot wound ($n = 13$), and other causes ($n = 34$).

Cotinine. Smoking data were not consistently available at the time of death. Thus, we classified smokers on the basis of serum cotinine. This might lead to the misclassification of some smokers as nonsmokers because of the 48-h half-life of cotinine. But because these individuals died unexpectedly (*e.g.*, trauma), it is unlikely that they changed their smoking patterns before death. Serum cotinine was determined by a RIA kit (STC Technologies, Bethlehem, PA) with modifications as reported (13). Subjects with a serum cotinine concentration >14 ng/ml were classified as smokers (14).

PAH Analysis. The GC-MS method was described elsewhere (12). Briefly, PAHs were extracted into hexane from 4 g of tissue after saponification. The extract was separated using a HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) on a Restek Rtx-5 MS 5% diphenyldimethylpolysiloxane column and analyzed by GC-MS HP 5973 mass spectrometer (Agilent Technologies) using selected ion monitoring mode. The GC-MS was calibrated by standard solutions containing all 11 target compounds at concentrations ranging from 0.5 to 50 pg/μl. The standard solutions also contained internal

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[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; BAP, benzo(*a*)pyrene; PAH, polycyclic aromatic hydrocarbon; FTC, Federal Trade Commission; GC-MS, gas chromatography-mass spectrometry; BA, benzo(*a*)pyrene; BBF, benzo(*b*)anthracene; BKF, benzo(*k*)fluoranthene; DA, dibenzo(*a*,*h*)anthracene; IP, indeno(1,2,3)pyrene; FL, fluoranthene; PY, pyrene; CH, chrysene; BEP, benzo(*e*)pyrene; BP, benzo(*g*,*h*,*i*)perylene.

Table 1 *PAH concentrations in human lung tissue (n = 70)*[a]

PAH	Median	Mean	Minimum	Maximum	SD
Noncarcinogenic PAHs					
BEP	0.007	0.016	ND[b]	0.321	0.052
BP	0.033	0.110	ND	1.783	0.293
CH	0.025	0.055	ND	0.645	0.105
FL	0.065	0.151	ND	1.574	0.250
PY	0.065	0.150	ND	2.162	0.293
Sum[c]	0.195	0.482	0.040	6.318	0.925
Carcinogenic PAHs					
BA	0.030	0.055	ND	0.691	0.102
BAP	0.036	0.086	ND	1.479	0.216
BBF	0.032	0.058	ND	0.540	0.094
BKF	0.017	0.039	ND	0.528	0.087
DA	0.009	0.053	ND	1.112	0.149
IP	0.026	0.088	ND	1.475	0.242
Sum	0.150	0.379	ND	4.837	0.756
Total PAHs[c]	0.345	0.861	0.071	11.155	1.659

[a] ng/g of wet tissue (ppb).
[b] ND, not detected.
[c] The sum of noncarcinogenic, carcinogenic, and total PAHs are calculated by adding all individual PAHs in a given category.

standards at fixed concentrations. Second-order calibration curves were fitted to this data using the least-squares method, weighting the data by the inverse of the concentration level to obtain an optimal fit at the lower levels.

Fat Content Analysis. Fat content was determined gravimetrically after extraction into hexane as described previously (15). The lung tissue (2 g) was homogenized with PBS (pH 7.2; 2 ml; Life Technologies, Inc., Rockville, MD) and methanol (2 ml; Sigma Chemical Co., St. Louis, MO). Hexane/ethyl ether (Sigma Chemical Co.), 10 ml of a 1:1 solution, was used to extract the homogenate overnight on a rotary mixer (Fisher, Inc., Fair Lawn, NJ). The organic layer was transferred to a new vial, the samples were extracted twice more for 2 h each as described above, and organic layers were pooled and dried under a constant stream of nitrogen.

Statistical Analysis. Statistical analyses were performed using STATISTICA '99 edition (StatSoft, Inc., Tulsa, OK) and SAS System for Windows version 8.01 (SAS Institute, Inc., Cary, NC). All of the PAH data entries are reported as ng/gram of wet tissue (ppb). The entries analyzed by GC-MS in duplicate were averaged. The background, average of 34 blank injections, was subtracted from each entry (12). PAH levels were summed for each individual, on a ng/gm wet tissue basis, where measurements below the detection limit were assigned zero values. PAHs known to be animal carcinogens (BA, BBF, BKF, BAP, IP, and DA; Ref. 1), and those that were not (FL, PY, CH, BEP, and BP) were summed separately. The distribution of the PAHs in the tissue was not normal but could be normalized with log transformation. To assess the effects of smoking on PAH levels, nonparametric methods (Mann-Whitney *U* test and Spearman R correlation coefficient) and logistic regression analysis were used (SAS System for Windows 8.01; SAS Institute Inc.). Dependent (PAH) variables were dichotomized by the median. Controlling variables for the logistic regression were gender, race, age, and fat content. Interactions between smoking and the other four independent variables (gender, race, age, and fat content) were evaluated. ANOVA with log-transformed PAH concentrations was used to assess the difference for PAH levels by smoking status, where nonsmokers were characterized by cotinine <14 ng/ml, and light smokers (cotinine 14–339 ng/ml) and heavy smokers (cotinine >339 ng/ml) were split by the median cotinine concentration among smokers (median, 339 ng/ml).

Results

The concentrations of PAH identified in lung tissue are shown in Table 1. About 45% of the detected total PAHs, on a ng/g basis, falls in the carcinogenic category. The relative mean abundance of the individual PAHs varied ~10-fold between the most (FL) and least (BEP) abundant PAH. The data show that none of the PAHs was detected in all of the tissues examined, but all of the PAHs were detected in at least some samples. Our analyses concentrated on the effect of smoking on PAH concentration in the lung tissue.

Using serum cotinine to classify smoking status, there were 37 smokers and 33 nonsmokers. As indicated by the Mann-Whitney *U* test, smoking increased the concentration of five PAHs (BA, BAP, BP, BKF, and IP; Table 2). Interestingly, four of six carcinogenic PAHs were statistically affected by smoking, whereas only one of five PAHs classified as noncarcinogenic was significantly increased. The sum of carcinogenic PAHs was therefore significantly higher in smokers ($P < 0.01$), whereas the sum of noncarcinogenic PAHs was not ($P = 0.12$). There was an overall agreement of the nonparametric and logistic regression analyses (Table 2), with odds ratios for the carcinogenic PAHs typically higher than the noncarcinogenic ones. The OR for the sum of the carcinogenic PAHs (OR, 8.20; 95% CI, 2.39–28.09) was >3-fold higher than the sum of the noncarcinogenic PAHs (OR, 2.61; 95% CI, 0.75–9.12). Interaction terms were found to be nonsignificant and were eliminated from the models. Colinearity was not detected in any of the models. Linear regression analysis of the data normalized by natural logarithm transform was also consistent with the above results (data not shown).

Subjects were divided by cotinine levels into nonsmokers, light smokers, and heavy smokers as described in "Materials and Methods" (Fig. 1). ANOVA using log-transformed data indicated a dose-response trend for increased PAH concentrations with increased smoking status. By this method, even the increase in noncarcinogenic PAHs reached significance (Table 3).

Differences between the African-American and Caucasian groups were not detected in the overall analysis of the lung samples (data not

Table 2 *Effect of smoking on PAH in lung tissue*

	Mann-Whitney *U* test[a]			Logistic regression[b]			
PAH	Smokers $n = 37$	Nonsmokers $n = 33$	P	Parameter estimate	OR for smoking	95% CI	P
Noncarcinogenic PAHs							
BEP	0.010	0.005	0.19	0.85	2.33	0.76–7.19	0.14
BP	0.040	0.025	0.04[c]	1.14	3.12	1.02–9.65	0.05[c]
CH	0.029	0.021	0.11	1.11	3.02	0.94–9.73	0.06
FL	0.093	0.059	0.43	0.39	1.48	0.49–4.46	0.48
PY	0.069	0.062	0.16	0.07	1.04	0.99–1.09	0.12
Sum	0.220	0.177	0.12	0.96	2.61	0.75–9.12	0.13
Carcinogenic PAHs							
BA	0.033	0.023	0.01[c]	0.98	2.66	0.87–8.19	0.08
BAP	0.051	0.025	<0.01[c]	1.91	6.80	1.94–23.95	<0.01[c]
BBF	0.036	0.027	0.13	0.92	2.51	0.84–1.08	0.09
BKF	0.020	0.009	0.01[c]	2.04	7.71	1.96–30.27	<0.01[c]
DA	0.017	0.000	0.09	0.99	2.71	0.84–8.67	0.09
IP	0.042	0.016	<0.01[c]	1.55	4.75	1.49–15.12	<0.01[c]
Sum	0.208	0.113	<0.01[c]	2.10	8.20	2.39–28.09	<0.01[c]
Total PAHs	0.44	0.31	0.01[c]	0.90	2.46	0.79–7.72	0.12

[a] Entries for Mann-Whitney *U* test are medians in ppb (ng/g of wet tissue).
[b] Logistic regression variables were dichotomized by their medians; see text for controlling variables (age, gender, race, and fat content).
[c] Significant difference.

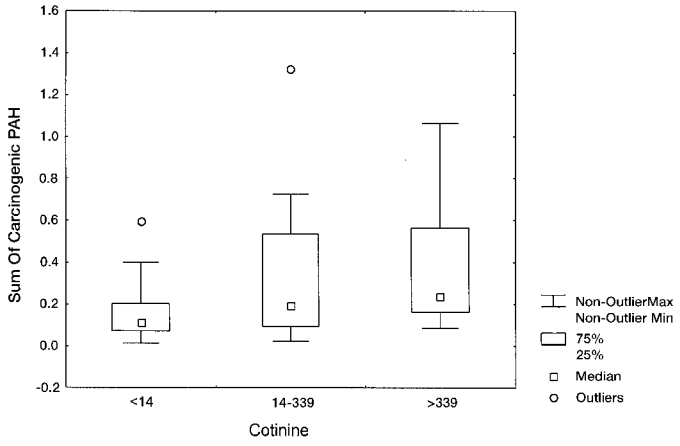

Fig. 1. Carcinogenic PAHs in nonsmokers, light smokers, and heavy smokers. Nonsmokers were defined by cotinine levels <14 ng/ml. The median value of smokers (cotinine, 339 ng/ml) was used to separate medium and heavy smokers. Three outliers (one for each category) are out of scale of the graph and are not represented for clarity.

Table 3 *Effect of increasing smoking status on PAH concentrations in lung tissue*

PAH	Median for smoking status[a]			
	Nonsmoker	Light	Heavy	P^b
Noncarcinogenic PAHs				
BEP	0.005	0.008	0.014	0.08
BP	0.025	0.035	0.081	<0.01[c]
CH	0.021	0.030	0.027	0.65
FL	0.059	0.052	0.099	0.08
PY	0.062	0.064	0.104	0.03[c]
Sum	0.177	0.209	0.381	0.03[c]
Carcinogenic PAHs				
BA	0.023	0.033	0.036	0.02[c]
BAP	0.025	0.033	0.054	0.01[c]
BBF	0.027	0.033	0.041	0.100
BKF	0.009	0.021	0.019	0.16
DA	ND[d]	ND	0.024	0.63
IP	0.016	0.037	0.044	0.01[c]
Sum	0.113	0.191	0.235	<0.01[c]
Total PAHs	0.313	0.389	0.735	<0.01[c]

[a] Light and heavy smoking status characterized by the median cotinine levels among smokers.
[b] ANOVA was used where PAH levels were log transformed, indicating a trend for increasing levels.
[c] Significant difference.
[d] ND, not detected.

shown). This was also true for the separate analysis of race in the subcategories of males and females, with the exception of BEP which was higher in Caucasian females than in African-American females ($n = 38$; $P = 0.049$). When the influence of race was analyzed separately for the subgroup of smokers/nonsmokers, DA was the only PAH significantly higher in African-American males compared with Caucasian males ($n = 32$; $P = 0.046$). It should be noted that the study does not have sufficient power to fully address the racial differences.

The concentration of PAHs in the lung tissue of males compared with females was uniformly higher for both the carcinogenic and noncarcinogenic PAHs (Table 4), although there were similar percentages of male and female smokers, and the cotinine levels tended to be higher for the females compared with the males (borderline statistical significance). Almost every individual PAH was higher in males compared with females. Interestingly, the increase in males compared with females was mostly attributable to the subcategory of African-Americans (Table 5).

The Spearman R coefficient was used to analyze the correlation of age with PAH concentrations. Age correlated with the sum of the noncarcinogenic PAHs ($r = 0.25$; $P = 0.03$) but not the sum of the carcinogenic PAHs ($r = 0.12$; $P = 0.32$). For individual PAHs, only the concentrations of FL ($r = 0.35$; $P = 0.002$), PY ($r = 0.29$; $P = 0.01$), and BA ($r = 0.28$; $P = 0.02$) were significantly correlated with age; but the correlation coefficients are relatively small, and the association could be attributable to chance.

The average fat content of the lungs was 1.1%, with a range of 0.1 to 5.7% (data not shown). We did not find a significant correlation of any PAH, or sum of PAHs, with the fat content of the lung tissue.

Discussion

The results demonstrate that smoking increases PAH concentrations ~2-fold, and those PAHs that are carcinogenic are present at higher concentrations. The risk for having higher carcinogenic PAHs compared with noncarcinogenic PAHs was >3-fold. Moreover, there was a dose-response relationship for smoking characterized by increasing serum cotinine. Men had a higher concentration of PAHs than women, although the smoking levels were equivalent. This was mostly explained by differences in African-Americans compared with Caucasians, although overall there was no difference between the races.

There have been several previous reports of PAH concentrations in tissues, but this study uses a more sensitive detection method, is more comprehensive, and is the first study to detect significant increases of BAP and other PAHs with smoking, including a dose-response relationship (16–19). These previous studies of PAHs in human tissues

Table 4 *Gender differences for PAHs in lung tissue*

PAH	Mann-Whitney *U* test[a]		
	Male ($n = 32$)	Female ($n = 38$)	P
Noncarcinogenic PAHs			
BEP	0.014	0.000	0.01[b]
BP	0.046	0.025	0.03[b]
CH	0.030	0.022	0.04[b]
FL	0.086	0.059	0.34
PY	0.084	0.056	0.09
Sum	0.246	0.164	0.02[b]
Carcinogenic PAHs			
BA	0.033	0.023	0.01[b]
BAP	0.051	0.025	0.01[b]
BBF	0.036	0.027	0.13
BKF	0.020	0.009	0.01[b]
DA	0.017	0.000	0.09
IP	0.042	0.016	0.01[b]
Sum	0.208	0.113	0.01[b]
Total PAHs	0.44	0.31	0.03[b]

[a] Entries are medians in ppb (ng/g of wet tissue).
[b] Significant difference.

Table 5 *Gender differences by race and smoking status for PAHs in lung tissue*

PAH	Mann-Whitney *U* test[a]		
	Male	Female	P^b
African-American nonsmokers			
Noncarcinogenic	0.370	0.140	0.02[b]
Carcinogenic	0.330	0.110	0.05[b]
Total	0.763	0.229	0.04[b]
African-American smokers			
Noncarcinogenic	0.419	0.192	0.35
Carcinogenic	0.465	0.174	0.02[b]
Total	1.013	0.327	0.08
Caucasian nonsmokers			
Noncarcinogenic	0.221	0.198	0.84
Carcinogenic	0.109	0.073	0.95
Total	0.346	0.271	0.95
Caucasian smokers			
Noncarcinogenic	0.220	0.158	0.39
Carcinogenic	0.208	0.204	0.77
Total	0.428	0.362	0.63

[a] Entries are medians in ppb (ng/g of wet tissue).
[b] Significant difference.

report values that vary by two orders of magnitude, possibly because of different population characteristics (race and smoking status), extraction/quantitation methods (UV-Vis spectroscopy, fluorescence, and mass spectrometry), and other methodological differences (wet or dry tissue weight). Importantly, the majority of previous studies used cancer patients, and cancer status was shown to affect PAH concentration in tissues (17).

The concentration of BAP in lung tissue has been reported most frequently. Lodovici *et al.* (16) reported BAP and five other PAHs in 20 lung autopsy tissues, where BAP concentration correlated with BAP DNA-adducts. Seto *et al.* (17) analyzed BAP, BKF, and BP in 364 lung samples from both cancer-free and cancerous formalin-fixed autopsies. The authors found that cancer tissue contains higher PAH concentration than noncancerous tissue, and that, similar to this study, males have higher PAH concentrations in the lung than females. Tokiwa *et al.* (18) analyzed BAP in 158 lung tumor resections and proposed a correlation of the PAHs with environmental exposure. Tomingas *et al.* (19) analyzed 12 PAHs in 33 bronchial carcinoma tissues but detected only four of the PAHs. Only BAP was found in all tissues at high concentrations in that study.

It is interesting that four of five PAHs that were increased in smokers were classified previously as carcinogenic to animals (1). The steady-state in the lung tissue is defined by complex exposure, distribution, and metabolism processes that cannot be unequivocally defined in this study. There are several possible explanations why smoking increases carcinogenic PAHs more that the noncarcinogenic PAHs. A smoking-related increase in noncarcinogenic PAHs such as FL and PY, which seem to accumulate in the tissue with age, is too small and cannot be detected. Another explanation is that metabolic turnover is an important determinant of the steady-state and that smoking affects clearance of the PAHs indirectly through induction of metabolic enzymes. Smoking could also be a better source of carcinogenic PAHs than diet and the environment. However, a conclusion about the source of PAH cannot be made without detailed knowledge of individual exposures because the concentration of PAHs in the environment and in cigarette smoke vary considerably, depending on several factors (source of environmental pollution, type of cigarette, and smoking topography; ref. 9). Because we do not know what the brand types were of the smokers, we could not determine PAH levels in relation to FTC tar yields, to assess whether lower yield cigarettes actually produced lower dose levels. PAH concentrations were consistently higher in males than in females with significant differences for several individual hydrocarbons. This might be attributable to differences in the type of cigarettes smoked by men compared with women. Men tended to smoke heavier cigarettes with higher tar content at the time when exposures occurred.

Lung cancer rates differ by gender and race. The highest rates are observed for African-American males (112/100,000), followed by Caucasian males (73/100,000) and African-American and Caucasian females (46 and 43/100,000 respectively; Ref. 20). The PAH concentration in lung tissue from this study follows the same trend, although the difference by race was not statistically significant. African-American males have the highest PAHs (0.784 ng/g), followed by Caucasian males (0.407 ng/g), African-American females (0.308 ng/g), and Caucasian females (0.279 ng/g). In this study, African-American males had the highest concentration of almost every PAH examined, with Caucasian males second in many cases. This ranking suggests that African-American males have higher doses compared with other groups, or that metabolism/distribution of the PAHs in African-American males is different. It is known, for example, that African-Americans smoke mentholated cigarettes more frequently than Caucasians (21), which could affect exposures and inhalation patterns.

Fat content did not correlate with the PAH concentration in the lung tissue. We had expected to find a correlation because PAHs are lipophilic. This indicates that the PAHs do not accumulate in the fat of the lung. This result is consistent with a previous study that reported low PAH content in human fat tissue (22). The concentrations (<1 ppb) are much lower than, for example, concentrations of some polychlorinated biphenyl compounds (>1 ppm), which were shown to accumulate in the fat. It was shown that only the polychlorinated biphenyls, which are not metabolized by cytochrome P-450, accumulate in the adipose tissue (23). The available information on the metabolism of PAHs in human lung suggests that the metabolic activity might be an important determinant of the steady-state of the PAHs in the tissue (24).

There are several limitations to this study. The use of cotinine as a marker of smoking is well accepted, and higher levels are indicative of greater smoking levels (14). However, cotinine is only a marker of recent smoking and does not reflect lifetime exposure, whereas the PAH levels might reflect long-term exposure. Also, some individuals might be misclassified as nonsmokers if they stopped smoking recently. In this study group, because most died unexpectedly from trauma or cardiac disease, it is unlikely that the subjects actually changed their smoking habits. Another limitation is the small size of the study, making it difficult to assess race and gender issues combined, although it was large enough to reveal overall patterns. Finally, we were unable to actually obtain lifetime smoking histories, brand use, and other factors that would affect exposure. This information would be needed for additional interpretations of the data.

In conclusion, this study shows that smoking increases the concentration of BAP and other carcinogenic PAHs in the human lung tissue with a dose-response relationship. These methods could provide for direct assessment of actual exposure to PAHs in target organs, rather than estimating exposure through FTC tar yields or self-reported histories. Also, additional data would be useful to study the impact of metabolic phenotypes and/or cigarettes with different FTC tar yields and the exposures from cigarette-like devices on the steady-state of PAH in the lung tissue.

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