Journal of Toxicology and Environmental Health, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713667303

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Online publication date: 08 April 2011

To cite this Article Saxena, Rajiv K. , McClure, Michael E. , Hays, Michael D. , Green, Francis H. Y. , McPhee, Laura J. , Vallyathan, V. and Gilmour, M. Ian(2011) 'Quantitative Assessment of Elemental Carbon In The Lungs of Never Smokers, Cigarette Smokers, and Coal Miners', Journal of Toxicology and Environmental Health, Part A, 74: 11, 706 — 715
To link to this Article: DOI: 10.1080/15287394.2011.556059
URL: http://dx.doi.org/10.1080/15287394.2011.556059

Journal of Toxicology and Environmental Health, Part A, 74:706–715, 2011

ISSN: 1528-7394 print / 1087-2620 online
DOI: 10.1080/15287394.2011.556059

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QUANTITATIVE ASSESSMENT OF ELEMENTAL CARBON IN THE LUNGS OF NEVER SMOKERS, CIGARETTE SMOKERS, AND COAL MINERS

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Inhalation exposure to particulates such as cigarette smoke and coal dust is known to contribute to the development of chronic lung disease. The purpose of this study was to estimate the amount of elemental carbon (EC) deposits from autopsied lung samples from cigarette smokers, miners, and control subjects and explore the relationship between EC level, exposure history, and the extent of chronic lung disease. The samples comprised three subgroups representing never smokers (8), chronic cigarette smokers (26), and coal miners (6). Following the dissolution of lung tissue, the extracted EC residue was quantified using a thermal-optical transmission (TOT) carbon analyzer. Mean EC levels in the lungs of the control group were 56.68 ± 24.86 (SD) μg/g dry lung weight. Respective mean EC values in lung samples from the smokers and coal miners were 449.56 ± 320.3 μg/g and 6678.2 ± 6162 μg/g. These values were significantly higher than those obtained from the never-smoker group. EC levels in the lung and pack-years of cigarette smoking correlated significantly, as did EC levels and the severity of small airway disease. This study provides one of the first quantitative assessments of EC in human lungs from populations at high relative risk for the development of chronic lung disease.

The relationships between cigarette smoking and cancer and between inhalation of coal dust and pneumoconiosis are well established (Bergman & Caswell, 1972; Shields, 2000; Smith et al., 2000; Vineis et al., 2004; Roller, 2009). Due to incomplete combustion, cigarette smoke contains a vast array of organic compounds, some of which are toxic or carcinogenic (Hoffman & Hoffman, 1997; Daisey et al., 1998; Fowles & Dybing, 2003). Cigarette smoke also includes a minor fraction of carbon black-like ultrafine elemental carbon (EC) particles that are refractory and possibly of toxicological concern (Daisey et al., 1998; Hildemann et al., 1991; Birch, 2003). Carbon black alone was shown under

Received 6 April 2010; accepted 14 November 2010.
We remember our dear friend and colleague Dr. V. Vallyathan, who passed away in an automobile accident during the preparation of this article. R. K. Saxena was supported through a senior fellowship of the U.S. National Research Council. F. H. Y. Green was supported through a grant from the Alberta Lung Association, Health Canada, and a contract with the U.S. National Institute of Occupational Safety and Health. This article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.
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"overload" conditions to induce tumors in rats (Nikula et al., 1995; Borm and Driscoll, 1996). Although a direct carcinogenic effect of carbon black per se has not yet been established in humans, many other direct and indirect health effects of carbon black have been reported (Baan, 2007; Morfeld & McCunney, 2007; Penn et al., 2005; Rausch et al., 2004; Soper et al., 2007; Sorhan & Harrington, 2007; Stampfli & Anderson, 2009). Carbon black can act as a carrier of carcinogenic organic compounds and may also provide an active surface for facilitating chemical changes of adsorbing organic species (Gerde et al., 2001; Oh & Chiu, 2009). EC is ubiquitous in the atmosphere due to anthropogenic combustion and biomass burning, but reliable estimates of the deposition of EC in the lungs of exposed humans are not readily available. Such data are required to better understand the extent of EC particle accumulation in the lungs and the fundamental aerosol EC dose-response relationship. In addition, if the organic carbon to elemental carbon (OC/EC) ratio of the complex aerosol is known, the EC measurement can be used as a tracer to predict the amount of OC that was deposited. The particular foci here were (1) to quantify the amount of EC in autopsied lungs of individuals who had been extensively exposed to EC in aerosol particles either occupationally or through smoking compared to nonsmoking controls, and (2) to explore the relationship with exposure history, where known, and disease severity.

Recently a sensitive technique was developed that combines tissue dissolution and liquid extraction methodology with thermal-optical analysis to quantify the intracellular concentration of diesel exhaust particles (DEP) in lung epithelial cells and macrophages in culture (Saxena et al., 2008). The technique took advantage of the fact that a consistent portion of the diesel exhaust particles comprised EC (Saxena et al., 2008). Since being established, the method has been further modified to investigate the accumulation of EC in the lungs of Wistar Kyoto and spontaneously hypertensive rats exposed to diesel engine exhaust (Saxena et al., 2009). In the present study the technique was modified and advanced further in an effort to assess the chronic accumulation of EC particles in the lungs of nonsmokers, smokers, and coal miners. In doing so, the applicability of the technique for autopsied human lung specimens representative of different degrees of EC particle exposure was tested.

MATERIALS AND METHODS

Subject Populations

Smokers and Never-smokers Samples of lung tissues were derived from two sources. The first were from a series of 139 unselected autopsies conducted at the Foothills Hospital from 1986 through 1990. The Foothills Hospital is a tertiary care teaching hospital in southern Alberta, Canada, serving an urban population of approximately 800,000 (at that time) and a rural population of approximately 200,000. The second source was from 70 control autopsy lungs obtained for a study of fatal asthma in the Prairie Provinces of Canada during 1992–1995 (Hessel et al., 1999). Both studies received approval from the University of Calgary Conjoint Ethics Committee. Consent for these studies was obtained from the next of kin, who completed a comprehensive (lifetime) questionnaire listing occupational and environmental exposures as well as a smoking history. The questions pertaining to the smoking history were based on a questionnaire developed by the American Thoracic Society (http://www.thoracic.org/statements/resources/archive/rrdquacer.pdf). This included information on smoking status and if a smoker, years of smoking, pack-years (number of packs smoked × years smoked), and smoking of pipes and cigars. In addition, data were collected on exposure to secondhand smoke in smokers and nonsmokers. The primary variable for cigarette smoking used in the analyses was pack-years, which is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. A sample of 34 cases was chosen at random for lung carbon analysis, of which

8 individuals were nonsmokers and 26 were smokers with varying numbers of pack-years. The overall severity of small airway disease for the respiratory bronchioles was graded as previously described (Schenker et al., 2009) on hematoxylin and eosin (H&E)-stained sections. The overall severity score was based on the average of individual scores for fibrosis, smooth muscle, inflammation, smokers' macrophages, and black pigment.

Coal Miners Samples of lung tissue were taken from six autopsied coal miners. The cases were part of a teaching collection at the University of Calgary illustrating coal workers' pneumoconiosis. Use of these cases for research and teaching had been obtained as part of the routine process for obtaining consent for autopsy from next of kin. The miners were also Alberta residents. No information on smoking or mining history was available for these cases. However, the samples, being selected for their teaching potential, were of excellent quality. Pathologic characterization was done as previously described (Kleinerman, 1979).

Selection of Samples for Lung Dust Analysis

Smokers and Never-Smokers Samples of lung parenchyma were taken from seven sites per lung: four from the upper lobe (EFGH) and three from the lower lobe (IJK) (Figure 1). The sites were taken to be representative of central and peripheral parenchyma. Approximately 10–20 g of formalin-fixed (wet) lung tissue from each sample site was placed into a clean 50-ml Nalgene container labeled by case number and site of sampling. Three to 4 g of tissue from each of the 7 sample sites was minced into small pieces and combined into a separate Nalgene container. This sample, which had 20–30 g of wet lung tissue, was considered to be representative of lung parenchyma as a whole. The sample did not include pulmonary lymph nodes. One- to 4-g aliquots of the minced homogenate of the lung tissues were used for the subsequent analyses.

Coal Miners Whole lung sections were available and sampled as already described for smokers and nonsmokers. In addition, the pneumoconiosis was graded as recommended by the Pneumoconiosis Committee of the College of American Pathologists (Kleinerman, 1979).

Wet/Dry Weight Ratios Aliquots of the wet lung samples taken as already described were blotted to remove excess fluid and weighed and then incubated in an oven at 60°C and for at least 3 d until the weight remained stationary. The ratio of the wet weight to the dry weight was recorded.

Processing of Lung Samples for Elemental Carbon Analysis

The samples were weighed and processed for carbon particle isolation as described previously (Saxena et al., 2008, 2009). Briefly, the chopped tissue was transferred into a 15-ml plastic tube containing 5 ml of Solvable (Perkin Elmer, Waltham, MA). The capped tubes were placed in a dry bath heater at 65°C for 24 h. The tube contents were then thoroughly dispersed and centrifuged at 80,000 × g for 25 min (Beckman Ultracentrifuge, SW 41 swinging bucket rotor). The resultant supernatant containing the dissolved tissue mass was discarded. The pellet containing EC particles was resuspended in 1 ml Solvable to repeat the heat digestion step (65°C × 24 h) and ensure completion of the tissue material dissolution. EC particles were then pelleted by Microfuge centrifugation at 16,000 × g for 25 min and the pellets were washed 2 times with 1-ml volumes of normal saline. In these studies, the final carbon pellet was suspended in 1 ml saline (1 mg NaCl/ml) and sonicated using a probe tip sonicator. A 50-μl aliquot drawn from the sonicated suspension was then transferred to a quartz filter paper punch (1 × 1.45 cm) and analyzed for elemental (EC) and organic carbon (OC) content. A Thermal-Optical Transmission (TOT) carbon analyzer running NIOSH method 5040 was used to obtain the quantitative EC speciation data (Birch, 2003). The EC term is often considered interchangeable with the

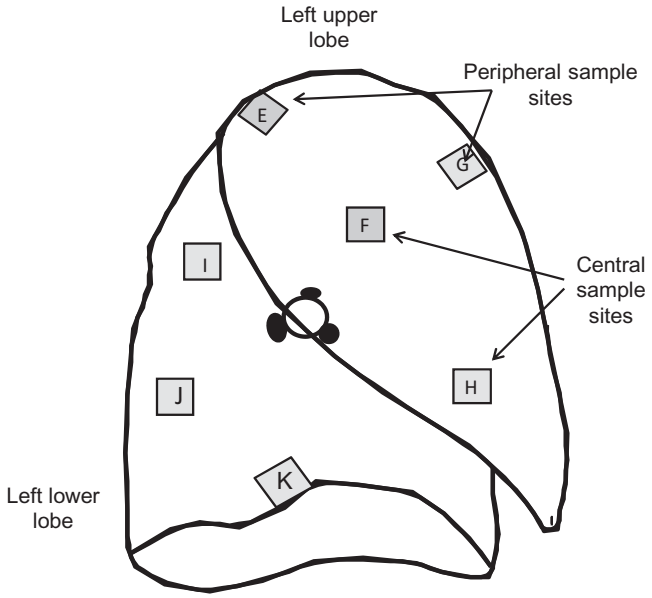

FIGURE 1. Diagram of saggital section of left lung to show sample sites for lung dust analysis.

terms "soot," "char," or BC. Here, EC is being operationally defined as the carbon mass that evolves in a He/O_2 environment during a set heating cycle following an OC char correction. The amount of EC was initially expressed as EC per gram wet lung. To account for variable amounts of water in the tissue samples the values were also normalized to EC per gram dry lung using the wet/dry tissue ratios.

Statistical Analysis

Comparison of carbon deposits in lungs of lifelong nonsmokers, cigarette smokers, and coal miners was conducted by analysis of variance (ANOVA). Correlation of carbon deposits with pack-years of smoking was estimated by nonlinear, dynamic-fit regression and/or Pearson product moment correlation analyses using Sigma Plot version 11.0 software. Several manual correlation calculations and Tukey HSD testing (JMP, Version 7.0, SAS Institute, Inc.) were independently performed. The criterion for significance was set at $p < .05$. Chi-squared tests and *T*-tests were performed using the PASW 17.0 software.

RESULTS

Characteristics of the Study Groups

The characteristics of the never-smokers and cigarette smokers are shown in Table 1. The cigarette smokers were older than the never smokers, and were more likely to have lived in a rural environment. The smokers were heavy consumers with a mean pack-years value of 45.6. The cigarette smokers displayed significantly more severe small airway disease than nonsmokers (Table 1). There was a significant correlation between pack-years smoked and the severity of the small airways disease. The type and grade of pneumoconiosis in the coal miners are shown later, in Table 5. Unfortunately, smoking and detailed mining information was not available for this group.

Elemental Carbon Deposits in the Lungs of Control Nonsmokers

Lung samples from 26 cigarette smokers, 8 nonsmokers, and 6 coal miners were analyzed in this study. For each sample, the total amount of EC was estimated as described in the Materials and Methods section. Knowing the wet weight of the sample taken and its

TABLE 1. Characteristics of the Never-Smoker and Cigarette-Smoker Cases

	Never smokers	Smokers
Gender (M/F)	4/4	21/5*
Age (yr) (mean ± SE)	42.8 ± 8.7	62.8 ± 2.4**
Pack-years (mean ± SE)	NA	45.6 ± 5.4
Urban/rural living (U/R)	8/0	22/4
Small-airway disease score	0.17 ± 0.08	1.48 ± 0.09***

Note. Significance indicated by $^{*}p = .002$ between male and female smokers (chi squared); $^{**}p = .056$ compared to never smokers (independent samples *T*-test); $^{***}p < .001$ compared to never smokers (independent samples *T*-test).

wet/dry weight ratio permitted the amount of EC per gram wet or dry weight to be computed. Mean values of EC (μg/g wet and dry weight) for these control samples were 11.37 ± 3.79 (SD) and 56.68 ± 24.86 (SD), respectively. Individual values and ranges are shown in Table 2.

Elemental Carbon Deposits in the Lungs of Smokers

Mean values for the EC (μg/g wet and dry weight) of the smokers samples were 73.50 ± 41.79 (SD) and 449.56 ± 320.3 (SD), respectively. These values were significantly higher than the values of EC deposits in control lungs. Individual values and ranges are shown in Table 3. Since we had access to the pack-years values for the smokers from whom the lung samples were derived, the relationship between the pack-years and EC accumulation was studied. The relevant pack-years values are depicted in Table 3. The range noted was 4 to 120. The mean value was 45.58 ± 27.74 (SD). The sampling breadth allowed a realistic range for analysis. In addition, Pearson correlation analyses (Table 4) demonstrated positive significant correlation coefficients for the following variable comparisons using the demographic information provided with the samples: age versus total EC (μg), age versus EC (μg per g dry (or wet) weight), years of smoking versus total EC (μg), years of smoking versus EC (μg per g dry (or wet) weight), pack-years versus total EC (μg), and pack-years versus EC (μg per g wet weight). These results demonstrated positive correlations for all but one comparison, pack-years versus EC (μg per g dry weight). An independent manual calculation for the correlation of pack-years and EC (μg per g dry weight) also failed to show a significant correlation or *p* value less than .05. As expected, mean values of EC per gram wet weight and EC per gram dry weight of lung from smokers and nonsmokers are significantly different according to an independent Tukey's HSD test, which in this case was more stringent that a *t*-test.The relationship between EC and small airway disease was explored. There were significant correlations between EC μg per g dry weight and overall small airway disease severity score ($r = .42$), airway wall fibrosis ($r = .41$), and airway wall black pigment ($r = .35$).

Elemental Carbon Deposits in the Lungs of Coal Miners

Mean values of EC (μg) per gram wet and dry weight in the six coal miner lungs were 890.3 ± 830.4 (SD) and 6678.2 ± 6162 (SD), respectively. These values for EC

TABLE 2. Elemental Carbon in Lungs of Community Never-Smokers

Subject number	Sample wet weight (g)	Total EC in sample (μg)	EC, μg/g wet weight	Wet/dry ratio	EC, μg/g dry weight
1	1.761	23.38	13.28	4.50	59.76
2	3.740	55.93	14.95	5.50	82.23
3	3.937	33.53	8.52	4.43	37.74
4	3.004	46.38	15.44	4.75	73.34
5	3.753	17.85	4.76	3.66	17.42
6	1.953	19.37	9.92	4.00	39.68
7	1.212	17.65	14.56	6.25	91.00
8	1.477	14.05	9.51	5.50	52.31

TABLE 3. Elemental Carbon in Lungs of Community Cigarette Smokers

Subject number	Sample wet weight (g)	Total EC in sample (μg)	EC, μg/g wet weight	Pack-years	Wet/dry ratio	EC, μg/g dry weight
1	3.602	87.47	24.28	106.00	4.21	102.22
2	2.623	53.10	20.24	35.00	4.43	89.66
3	1.721	213.87	124.27	64.00	4.00	497.08
4	3.410	56.73	16.64	45.00	3.50	58.24
5	4.497	523.30	116.37	120.00	4.38	509.70
6	3.223	145.44	45.13	29.00	6.75	304.63
7	2.821	52.74	18.70	4.00	4.00	74.80
8	1.914	58.02	30.31	88.00	11.57	350.69
9	3.011	45.51	15.11	24.00	4.55	68.75
10	0.860	51.60	60.00	49.00	9.67	580.20
11	0.919	75.35	81.99	14.00	5.00	409.95
12	0.471	46.60	98.94	32.00	7.00	692.58
13	0.407	46.50	114.25	56.00	5.50	628.38
14	1.304	207.40	159.05	43.00	4.56	725.27
15	0.830	70.10	84.46	46.00	5.25	443.42
16	0.816	44.10	54.04	38.00	3.66	197.79
17	1.213	56.55	46.62	20.00	4.33	201.86
18	0.472	28.55	60.49	55.00	3.67	222.00
19	0.592	77.75	131.33	24.00	8.00	1050.64
20	0.388	26.90	69.33	60.00	4.37	302.97
21	0.537	56.45	105.12	76.00	8.00	840.96
22	0.726	79.40	109.37	36.00	9.50	1039.02
23	0.875	85.60	97.83	25.00	11.33	1108.41
24	0.431	42.60	98.84	51.00	4.71	465.54
25	0.454	47.70	105.07	20.00	6.00	630.42
26	0.660	15.40	23.33	25.00	4.00	93.32

TABLE 4. Summary of Comparison Significance of Smokers and Never Smokers

Variable for Pearson product moment correlation	Correlation coefficient (*r*)	*p* Value
Age vs. total EC μg in sample*	.373	.0298
Age vs. EC μg/g dry weight of sample*	.353	.0404
Age vs. EC μg/g wet weight of sample*	.506	.00229
Years smoking vs. total EC μg in sample*	.462	.006
Years smoking vs. EC μg.g wet weight of sample*	.596	.000202
Years smoking vs. EC μg/g dry weight of sample*	.438	.00956
Pack-years vs. total EC μg in sample*	.566	.000486
Pack-years vs. EC μg/g wet weight of sample*	.466	.00823
Pack-years vs. EC μg/g dry weight of sample**	.316	.0683
Sample EC μg/g wet weight vs. EC μg/g dry weight*	.869	2.59*E*-11

Note. Definitions ($n = 34$ for all cases): Positive correlation (*) = The pair(s) of variables with positive correlation coefficients and *p* values below .050 tend to increase together. For the pairs with negative correlation coefficients and *p* values below .050, one variable tends to decrease while the other increases. For pairs with *p* values greater than .050, there is no significant relationship between the two variables. No correlation (**) = There are no significant relationships between any pair of variables in the correlation table ($p > .050$).

were significantly higher than in never smoker and cigarette smoker lungs. The relationship between EC and grade of pneumoconiosis is shown in Table 5. Coal miners with the more severe categories of pneumoconiosis (progressive massive fibrosis or PMF) had greater EC than miners with simple coal workers pneumoconiosis. Individual values and ranges of EC (μg)/g wet or dry weight of the samples are given in Table 4.

TABLE 5. Elemental Carbon in Lungs of Coal Miners

Subject number	Wet weight of sample (g)	EC in sample (μg)	EC,μg/g wet weight	Wet/dry ratio	EC,μg/g dry weight	Pneumoconiosis: type and severity
1	1.002	113.3	113.1	3.763	425.5	Simple CWP, macules Severity: mild
2	1.387	2382.0	1718.0	7.000	12,026.0	PMF, macules, nodules Severity: moderate
3	2.128	2706.0	1271.7	9.857	12,535.1	PMF, macules, nodules. Severity: severe
4	0.979	56.5	57.7	6.667	384.8	Simple CWP, macules Severity: mild
5	0.989	300.0	303.3	8.187	2483.4	Simple CWP, macules, nodules Severity: moderate
6	1.073	1911.5	1878.0	5.950	11,174.1	Simple CWP, PMF Severity: severe

Note. CWP = coal workers' pneumoconiosis. PMF = progressive massive fibrosis.

DISCUSSION

The results indicate a significant accumulation of EC particles in the lungs of smokers that markedly exceeded control levels, and demonstrate a positive correlation with the extent of smoking. We observed that the accumulation of carbon particles in coal miners' lungs was substantially greater compared to smokers. In addition we show that the EC in the lungs of cigarette smokers correlates with the severity of small airway disease.

Cigarette smoke contains a multitude of particle-phase organic compounds, some of which are carcinogenic (Hoffman & Hoffman, 1997; Daisey et al., 1998; Fowles & Dybing, 2003). Zaebst et al. (1991) showed that roughly 98.2% w/w of the total carbon in cigarette smoke particles was in the form of organic carbon (OC), with the rest (1.8% w/w) being EC. Hildemann et al. (1991), Daisey et al. (1998), and Birch (2003) reported that EC comprises less than 1% of the cigarette smoke particles analyzed in their studies. Despite the relatively low EC concentration measured in cigarette smoke, it is known that large amounts of black carbonaceous pigment or tar deposit and accumulate in the lungs of longtime smokers (Pinkerton et al., 2000; Schenker et al., 2009; Fujiki et al., 2004). The present study indicates that the EC content in suspended cigarette smoke particles may serve as a surrogate of smoke inhalation exposures in humans, provided other sources of airborne elemental carbon are taken into account. EC is generally treated as a single refractory chemical entity that is amorphous and nonreactive, and measurement of EC is simple compared with the daunting task of measuring mixtures of complex tar-like substances. Our results show that the level of EC deposited in the lungs of nonsmokers minimally exposed to airborne carbonaceous particles averaged about 57 μg/g dry weight. This base level of EC is plausibly derived from passive exposures to EC inairborne particles produced from anthropogenic or pyrogenic combustion sources. Among others, secondary cigarette smoke, automobile and industrial exhausts, and biomass burning are examples of typical EC-producing emissions sources to which humans are regularly exposed.

Cigarette smoking resulted in a significant accumulation of EC in the lungs that was, on average, six- to sevenfold greater than the corresponding accumulation of EC in the lungs of the nonsmokers. Within the smoker group, EC measured in the lung correlated significantly to pack-years and to the severity of

small airway disease. The age of the cigarette smoker and their extent of exposure to workplace and other environmental dusts and fumes likely accounted for much of the variability in the levels of EC in the lung. Review of the questionnaire data from the smokers and nonsmokers revealed that proportionally more smokers than nonsmokers were employed in blue-collar occupations.

The concentration of EC in the lungs at death presumably reflected the balance between particle deposition and clearance over the lifetime. Recently Saxena et al. (2009) utilized an animal model to confirm that rates of diesel exhaust particle clearance in the lungs can vary. Furthermore, variables such as age, genetic disposition, and different local exposures to chemically transformed source emissions in the atmospheric environment all likely modulate EC levels in the lungs of each individual examined in this study, explaining the wide breadth of values observed. Larger studies, with greater power, will be required to explore the role of these other variables in the accumulation of EC in the lung.

The Pearson statistical comparisons provided additional confidence in the biological plausibility of the demographic correlations observed. It was not unexpected that age and years smoking would show correlations to micrograms EC per gram total sample (and wet or dry weight) of the samples if one assumes that there is an enhanced deposition of EC over time. Indeed, it would have been surprising had they not done so. The one discordance noted within the overall set of comparisons was for the Pearson statistic for the pack-year comparison group, in which no correlation was noted for pack-years and micrograms EC per gram dry weight of sample. This was interesting since the other two components of pack-years (age and years smoking) did show a positive correlation. It was noted that the age comparison to micrograms EC per gram dry weight of sample had a p value of .04 and the pack-years comparison to micrograms EC per gram dry weight of sample had a p value of .068, both of which are close to the cutoff value, but from opposite sides. It is possible that the small sample size and a limited robustness of the Pearson statistical power level may be a factor in this outcome. Given that "dry weight" is the sample status furthest downstream in the sample processing protocol, an initial concern was that the steps taken in converting wet weight samples to dry weight samples might introduce additional variability to the results. This concern was somewhat relieved by observing the reliable correlation of micrograms EC per gram wet weight and micrograms EC per gram dry weight of sample. It is possible, however, for the Pearson statistic that an increase in the distribution of variability within the dry weight samples is a factor in the discordance noted.

The levels of EC deposited in the lungs of coal miners were found to be markedly higher (14- to 17-fold greater) than those in the lungs of the smokers. Most interestingly, there were large variations of EC levels in the sample of coal miners' lungs that were related to the severity of their pneumoconiosis. Unfortunately, there were no records on duration of mining or smoking history for these miners; thus, our conclusions are tempered by these limitations. Other factors including variations in the rate of clearance of different carbon particles types from lungs could also contribute to the extent of accumulation of EC in lungs. Irrespective of these variations, a somber realization arises from considering that the total accumulation of EC in the lungs of the coal miners in this study amounted to several grams in some cases. These values are similar to those reported for coal dust in the lungs of autopsied coal miners from the United States, using gravimetric analysis (Sweet et al., 1974).

EC is a constituent of concern in cigarette smoke and biomass smoke, both of which are associated with increased chronic obstructive pulmonary disease (COPD) risk (Grigg, 2009). A recent study of infants living in New York found a significant association between ambient EC and cough during the cold and flu season that was not found for small airborne particulate matter ($PM_{2.5}$) (Patel et al., 2009). Although in the rat model carbon black particles were shown to induce tumors, similar experiments using other species have been

inconclusive (Nikula et al., 1995; Borm & Driscoll, 1996). While the biological consequences of EC deposits in human lungs are uncertain, EC particles may behave as "work platforms," carrying and (via catalysis) producing molecules with potentially adverse health effects (Gerde et al., 2001; Penn et al., 2005). This is of special relevance to cigarette smokers because cigarette smoke contains EC and a vast array of organic compounds likely to modulate biological processes (Daisey et al., 1998; Fowles & Dybing, 2003). For example, Oh and Chiu (2009) have recently reported accelerated reduction of 2,4-dinitrotoluene (DNT) to 2,4-diaminotoluene by dithiothreitol in the presence of carbon black. This observation opens up the possibility of EC acting as a catalytic bed for the in situ modification of certain organic compounds in lungs and also implies potential for indirect adverse health effects due to EC being present in the lungs.

In conclusion, data showed a relatively low basal level of EC in control healthy lungs and a significantly increased EC level in cigarette smokers. In smokers, the correlations observed between the EC level and pack-years and EC level and small-airway disease are convincing. Furthermore, the EC deposits in the lungs of coal miners were higher relative to those in nonsmokers at the base level. Further investigation of the adverse health consequences due to the presence of EC particles in lungs is warranted.

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