Small particles disrupt postnatal airway development

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Small particles disrupt postnatal airway development. J Appl Physiol 109: 1115–1124, 2010.—Increasing numbers of epidemiologic studies associate air pollution exposure in children with decreased lung function development. The objective of this study was to examine the effects of exposure to combustion-generated fine [230 and 212 nm number mean aerodynamic particle diameter (NMAD)] to ultrafine (73 nm NMAD) particles differing in elemental (EC) and organic (OC) carbon content on postnatal airway development in rats. Neonatal Sprague-Dawley rats were exposed from postnatal day 7 through 25, and lung function and airway architecture were evaluated 81 days of age. In a separate group of rats, cell proliferation was examined after a single particle exposure at 7 days of age. Early life exposure to 73 nm high OC/EC particles altered distal airway architecture and resulted in subtlety changes in lung mechanics. Early life exposure to 212 nm high OC/EC particles did not alter lung architecture but did alter lung mechanics in a manner suggestive of central airway changes. In contrast, early life exposure to 230 nm low OC/EC particles did not alter lung architecture or mechanics. A single 6-h exposure to 73 nm high OC/EC particle decreased airway cell proliferation, whereas 212 nm high OC/EC particles increased it and 230 nm low OC/EC particles did not. The early life exposure to ultrafine, high OC/EC particles results in persistent alterations in distal airway architecture that is characterized by an initial decrease in airway cell proliferation.

lair function; lung architecture; children; cell proliferation; development disruption

AIR POLLUTANTS, including particulate matter and ozone, have been shown to adversely affect human health. Epidemiologic studies suggest that these health effects can begin at birth; chronic exposure to air pollution is associated with reduced lung function growth in humans (2, 13, 14, 16, 22, 27, 42, 45, 51). Elevated particulate pollution has also been associated with increased medication use and hospital admission in children and adults. Children who exercise outdoors in areas with high levels of air pollution have a greater risk of developing asthma (15, 44). These observations are consistent with the possibility that particulate matter (PM) exposures can inhibit lung growth and pulmonary function. Small particles in the 100–200 nm size range are believed to play a causal role in decreased lung function among children living in areas with high traffic. Studies have shown that small particles can be carriers for metals and organic carbon compounds because of their small size and large surface area, increasing oxidative stress, inflammation, and impaired cellular defense (32, 36, 39).

While there have been numerous studies that describe alterations in pulmonary function and exacerbation of respiratory disease in response to particulate matter in human populations, there are few mechanistic studies in animal models. Some parallels can be drawn between particulate matter effects and studies of the effects of tobacco smoke, a complex mixture that also contains abundant particles, on lung growth and pulmonary function. A recent study of perinatal exposure to sidestream tobacco smoke in the rhesus monkey found decreased alveolar number and size and an increase in respiratory bronchial volume (1). Another study in mice found enhanced methacholine responsiveness later in life (56). The lungs of mice chronically exposed perinatally to traffic-related PM are less alveolarized than control (34). However, mice exposed only postnatally did not have changes in their alveoli. In one of the few studies to examine the conducting airways, rats exposed to sidestream smoke in the postnatal period were found to have accelerated airway epithelial differentiation (24). These studies suggest that the early period of lung development is a window of high susceptibility for lung damage due to air pollutants, but there are still limited in vivo studies that examine postnatal exposure to particles (12, 34, 35).

Proximity to freeways and other combustion emissions is associated with reduced lung function and greater preponderance of asthma in children (16, 51). Given the large number of parameters that affect engine emissions and the lack of engine emissions standards, comparison of toxicity studies is difficult. Laboratory-based combustion methods have the benefit of producing controllable, well-characterized, and repeatable PM for exposures that model ambient combustion sources (8, 17, 53). This is especially critical for studies that involve lung development where a stable, replicable environment is necessary. Furthermore, laboratory-based methods can be manipulated to produce different and controlled particle compositions.

In this study, we investigated the effects of postnatal exposure of male Sprague-Dawley rats to three combustion-generated small particles (19) differing in size and elemental (EC) and organic (OC) carbon content. We employed laboratory-generated combustion particles since they mimic particles emitted by real-world combustion sources but lack metals. We chose two particle sizes (near 70 and 200 nm) due to differences in their diffusive properties and two different composition regimens (high and low OC/EC ratio) to explore whether the effects observed are due to the organic or elemental carbon content. Cell proliferation related gene and protein expression were examined after a single exposure at 7 days of age. Airway
architecture and mechanics were examined in 81-day-old rats that were exposed for 19 days starting at 7 days of age.

**MATERIALS AND METHODS**

**Particle generation.** Particles were generated by an annular tubular burner that can be run in different modes to generate a variety of environmentally relevant particle types. In diffusion flame mode, the particle concentration and properties were controlled by varying ethylene fuel flow rate between 0.22 and 0.25 l/min with a surrounding coflow of 30 l/min of clean air. In premixed flame mode, an ethylene, oxygen, and argon mixture flowed through the 0.71-cm inside diameter center of the burner stabilized by an outer annulus of oxygen. Flow rates of each gas were calculated to achieve a total flow of 2 l/min while maintaining an estimated adiabatic flame temperature of 1,900 K. Gas flow rates for ethylene, oxygen, and argon were metered by mass flow controllers with a full scale accuracy of 2%, and typical flows were at 50% of full scale. Air and nitrogen flows were metered by rotometers. Equivalence ratios, φ, of 2.2 or 2.5 varied the elemental carbon to organic carbon (EC/OC) ratio. The flame was surrounded by a nitrogen jacket flowing at 10 l/min to prevent oxidation of the particles. For both modes, a three-way automobile catalyst oxidized carbon monoxide and hydrocarbons to carbon dioxide and reduced oxides of nitrogen to nitrogen and oxygen. All flame products from both diffusion and premixed flames were then mixed with HEPA/chemical bacteriological radiological (CBR)-filtered air. The diffusion flame produced a concentration of 2.4×10^8 particles/cm³ in the exposure chamber with a number mean aerodynamic particle diameter (NMAD) of 230 nm and a mass concentration of 71.7 μg/m³; we refer to these as DFP230. The premixed flames with φ = 2.2, 2.5 produced 9.5×10^4, 4.3×10^4 particles/cm³ in the chamber with a diameter (NMAD) of 72.7, 212.0 nm and a mass concentration of 20.0, 67.4 μg/m³, respectively, which we refer to as PFP73 and PFP212 (Table 1).

To analyze the three particle types for their EC and OC content, particles were sampled simultaneously from exposure and filtered-air chambers twice weekly onto Pallflex Tissuquartz filters. DFP230 and PFP212 were sampled for 180 min/filter and PFP73 for 240 min/filter. The filters were analyzed by a Sunset Labs OC/EC instrument (see Table 1). Organic carbon content in the FA exposures is likely due to dander and food particles in the chamber. The lack of contamination by outside air is confirmed by the low EC values. The mass concentrations used in this study are higher than typical ambient PM2.5 values: since rats clear PM much faster than humans, for chronic studies in the rat higher exposure concentrations are required to simulate the retained particle burden that builds up over years of human exposure.

**Animal exposures.** Litters of Sprague-Dawley rat pups with a lactating mother were delivered from the vendor (Harlan Laboratories) and were housed in filtered air chambers in American Association for Accreditation of Laboratory Animal Care-approved facilities. All procedures were part of an Institutional Animal Care and Use Committee-approved animal protocol. Male pups housed with lactating mothers were placed in filtered air chambers at age 1 day. For the acute responses, neonatal rats were exposed at 7 days of age for 6 h. For the chronic responses, exposures for 6 h/day, 5 day/wk for 19 days began when the pups were 7 days old and ended at 25 days old. At 21 days of age, the rat pups were weaned and transferred into an open wire mesh rodent inhalation cage module with two animals per cage. An age-matched set of pups was exposed to filtered air (FA) using the same protocol. At 28 days old, the animals were transferred from the exposure chambers to HEPA-filtered enclosures where they matured until study at 80–81 days of age when pulmonary function tests and lung casting for airway architecture analysis were conducted. Food (Purina rodent chow) and microfiltered deionized water were provided ad libitum. The animals were exposed to 12 h of light from 7:00 AM to 7:00 PM. Figure 1 shows a flow chart of the study. Note that we used different rats for the pulmonary mechanics and the architecture evaluations because airway casts from the rats after methacholine challenge (see *Pulmonary mechanics in METHODS*) substantially altered airway architecture.

**Casting procedures.** Animals were killed with an injection of pentobarbital sodium administered at a dosage of 0.5 ml/kg body wt. Lungs were fixed in chest via tracheal cannula with Karnovsky’s

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**Table 1. Exposure particle characteristics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter, nm</th>
<th>Concentration, μg/m³</th>
<th>OC, μg/m³</th>
<th>EC, μg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFP73</td>
<td>72.7</td>
<td>20.0</td>
<td>Expose</td>
<td>FA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.46</td>
<td>3.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.37</td>
<td>0.00</td>
</tr>
<tr>
<td>PFP212</td>
<td>212.0</td>
<td>67.4</td>
<td>Expose</td>
<td>FA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.16</td>
<td>6.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.62</td>
<td>0.47</td>
</tr>
<tr>
<td>DFP230</td>
<td>230.3</td>
<td>71.7</td>
<td>Expose</td>
<td>FA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.72</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>57.47</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Diameter is number median aerodynamic diameter (NMAD). OC and EC were averaged from 6 time measurements. FA, filtered air.
fixative at 30 cmH\textsubscript{2}O pressure for 1 h and then removed from the chest and stored in fixative. Fixed lungs were placed in buffered saline prior to casting. Silicone RTV was introduced to the lung through the trachea under a slight negative pressure (\text{-}80 \text{mmHg}) until it reached the distal airways. The silicone RTV was allowed to cure for 48 h, after which the airway tissue was removed with bleach (details in Ref. 28).

Table 2. BW and TLC normalized by BW in the different study groups at age of 80–81 days

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Body Weight, g</th>
<th>TLC/BW, ml/100 g</th>
<th>Number of Casts</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>365 ± 30 (74)</td>
<td>3.173 ± 0.047 (27)</td>
<td>32</td>
</tr>
<tr>
<td>PFP73</td>
<td>358 ± 22 (22)</td>
<td>3.358 ± 0.078 (10)</td>
<td>6</td>
</tr>
<tr>
<td>PFP212</td>
<td>375 ± 17 (16)</td>
<td>3.146 ± 0.083 (8)</td>
<td>9</td>
</tr>
<tr>
<td>DFP230</td>
<td>362 ± 29 (12)</td>
<td>3.209 ± 0.083 (10)</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of subjects in parentheses. No significant change between exposed and FA groups. BW, body weight; TLC, total lung capacity.

Acquisition of CT data. Lung casts were imaged using a commercially available microCT scanner, MicroCAT II (Siemens, Knoxville, TN) in high-resolution mode. The image was reconstructed using the Feldkamp reconstruction algorithm as a 768×768×1,000 array with corresponding voxel size of 0.053 mm×0.053 mm×0.053 mm. Image resolution was 43 \mu m (28).

Architecture extraction. Custom software was employed to extract branching patterns of conducting airways, airway diameter, length, branching angle, rotation angle (relative angle between bifurcations), as well as connectivity between airways from CT images of lung casts (28, 29). Branching angle was defined as the angle between parent branch and its daughter branch. Rotation angle was defined as the angle between successive bifurcation planes. Bifurcation plane is defined by a parent branch and its two daughters. Briefly, a geometric model of an airway bifurcation was fit to the CT image data by minimizing the distance between the model and the airway image data. With the use of computerized analysis of lung cast CT images, most conducting airways were measured and the total number of airways measured averaged \text{4,200} per cast. Airways were excluded from further analysis for two reasons: 1) the error normalized by

Fig. 2. Airway diameter (means \pm SD) as a function of generation number in the different groups from generation 0 to 10 (A) and from generation 11 to 22 (B). *P value <0.05, x indicates marginally insignificant with P value between 0.05 and 0.06, and &P value <0.0022 (Bonferroni adjustment). Log y-axis in (A), linear in (B).
airway radius was >25% or 2) the airway radius was smaller than two voxels (28).

**Pulmonary mechanics.** Rats were deeply anesthetized with an intraperitoneal injection of α-chloralose (2.5 g/100 ml) and urethane (25 g/100 ml) at a dose of 6 ml/kg. A midline incision was made over the cervical trachea following administration of topical anesthesia, and an endotracheal tube was placed into the trachea just caudal to the larynx. Once the rats were instrumented, the endotracheal tube was connected to a constant-volume ventilator/plethysmograph (FlexiVent4, SCIREQ) with a breathing frequency of 90 breaths/min, volume adjusted to body weight at 10 ml/kg, and a positive end-expiratory pressure of 2 cmH2O. Rats were then paralyzed with 0.05 ml intraperitoneal succinyl choline (20 mg/ml). Rats received a single airway challenge of saline aerosol for 30 breaths followed by 3 min of measurement of airway input impedance. Rats then received doubling doses of methacholine (0.0625–64 mg/ml) aerosol for 30 breaths followed by 3 min of evaluation of airway input impedance. Methacholine (250 μl) is added to the nebulizer for each dose that is given. Airway challenges were stopped when airway resistance doubled. Immediately following the procedure, animals were killed by an overdose of pentobarbital sodium.

Prior to and after each methacholine challenge, total lung capacity (TLC), input respiratory impedance (ZI), dynamic respiratory system resistance (Rr), and dynamic compliance (Cdyn) were measured. The input impedance data were fit to a constant phase model (20, 21) and estimates of airway resistance (Rn), respiratory system inertance (I), tissue damping (G), and tissue elastance (H) were derived. Tissue damping is closely related to tissue resistance and reflects the energy dissipation in the lung tissue, while tissue elastance reflects the energy conservation in the lung tissue.

**Airway-specific proliferating cell nuclear antigen expression.** Lungs were collected 24 h after a single 6 h acute exposure. The trachea was cannulated and the lungs were removed and inflated to capacity with RINalater (Ambion/Applied Biosystems, Foster City, CA). The lungs were stored at 4°C in RINalater for 24 h then moved to −20°C until microdissection and RNA isolation could be performed. Airways were microdissected from surrounding parenchyma (3) to yield an intrapulmonary airway specific lung fraction. RNA was isolated from airways using the RNeasy Plus Mini kit (QIAGEN, Valencia, CA) with gDNA eliminator columns. RNA (1 µg) was reverse transcribed into single-stranded cDNA using Multiscribe reverse transcriptase (Applied Biosystems). A StepOnePlus (Applied

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**Fig. 3.** Airway length (means ± SD) as a function of generation number in the different groups from generation 0 to 10 (A), from generation 11 to 22 (B). *P value <0.05, × indicates marginally insignificant with P value between 0.05 and 0.06, and &P value <0.0022 (Bonferroni adjustment). Log y-axis in (A), linear in (B).
Biosystems) was used for qRT-PCR. Each reaction contained 20 ng cDNA, 1 μl TaqMan Gene Expression Assay (GAPDH Rn99999916_s1 or PCNA Rn00673588_mH), 10 μl TaqMan Fast Universal PCR Master Mix, No AmpErase UNG, and RNase free water to make a final volume of 20 μl. GAPDH was used as a housekeeping gene. Fold change in gene expression in microdissected airways from animals (n = 4–10) per time point was calculated using the comparative Ct (2−ΔΔCT) method (33) and is expressed relative to the filtered air control.

For immunohistochemistry, lungs were fixed with paraformaldehyde at 30 cm of constant pressure through the tracheal cannula. Lung pieces were embedded in paraffin and sectioned at 5 μm. Hot citrate buffer was used for antigen retrieval. Endogenous peroxidase was blocked with a 10% peroxide solution and nonspecific binding was blocked with IgG-free bovine serum albumin (Jackson Immunoresearch Laboratories, West Grove, PA) for 30 min at room temperature. Sections were immunostained using the manufacturer’s suggested procedure with an avidin-biotin-peroxidase ABC kit (Vector Labs, Burlingame CA) and a monoclonal anti-PCNA antibody (DAKO, Carpinteria, CA) at 1:600 overnight. The signal was detected with 3’,3’-diaminobenzidine tetrahydrochloride. Controls included the substitution of primary antibody with phosphate buffered saline (5, 46, 57).

Statistics. Airway architecture data and cell proliferation assay were assessed for significance using a Student’s t-test, with Bonferroni correction where appropriate. Pulmonary mechanics data were analyzed using a multivariate general linear model (SPSS) with Tukey’s honestly significant difference as a post hoc test to identify significant differences between each particulate exposure group and the filtered air group for each parameter.

RESULTS

TLC normalized by body weights and body weight itself were similar between FA group and exposed groups (P = 0.8, 0.277, 0.076 for DFP230, PFP73 and PFP212, respectively; Table 2) at age of 80 or 81 days.

Airway architecture. Figures 2–5 show the generation-averaged airway diameter, length, branching angle, and rotation angle. There were no significant differences in airway diameter and length between the FA and DFP230 or PFP212.

![Fig. 4. Branching angle (means ± SD) as a function of generation number in different groups from generation 0 to 10 (A), from generation 11 to 22 (B). *P value <0.05, ×indicates marginally insignificant with P value between 0.05 and 0.06, and &P value <0.0022 (Bonferroni adjustment). Log y-axis in (A), linear in (B).](image-url)
Both generation-averaged airway diameter and length for the PFP73-exposed group were consistently smaller than FA. Diameter for PFP73-exposed animals was significantly smaller ($P < 0.05$) than FA in 9 of 23 generations and marginally insignificant in two additional generations ($0.05 < P < 0.06$); the differences were significant in several distal generations after Bonferroni adjustment ($P < 0.0022$) for multiple comparisons (Fig. 2). Length also decreased significantly in 7 of 23 generations (Fig. 3). Differences in the average diameter and length from FA were largest in the several distal generations (Figs. 2, 3). In generations 11–22, the average diameter and length decreased ~9% and 13%, respectively, due to PFP73 exposure.

PFP73 exposures elicited changes in branching and rotation angles between exposed and FA groups. Branching angle decreased in most generations (Fig. 4). Rotation angle also decreased in most generations and the differences from FA were significant in the middle generations (Fig. 5). The decrease in branching angle was significant in 6 of 23 generations and decrease in rotation angle was significant or marginally insignificant in five generations. The difference was statistically significant in generation 12 even after Bonferroni adjustment ($P < 0.0022$).

**Pulmonary mechanics.** Exposure to PFP212 resulted in a significant increase ($P = 0.013$) in the methacholine EC200 (Table 3), indicating decrease in airway responsiveness in this
group. Exposure to DFP230 or PFP73 did not significantly alter airway responsiveness to methacholine challenge compared with FA (Table 3). Exposure to PFP212 resulted in a significant increase in $R_s$ ($P = 0.005$) and $I$ ($P = 0.001$) compared with FA exposure (Table 3). In contrast, exposure resulted in a significant decrease in $I$ in the PFP73 ($P = 0.001$) group compared with FA. In addition, exposure to PFP230 resulted in a significant increase in $H$ compared with FA exposure (Table 3; $P = 0.002$).

PCNA expression. Proliferating cell nuclear antigen (PCNA), a gene associated with cell proliferation was quantitatively assayed using RT-PCR. Gene expression significantly decreased in neonatal rat airways following a single 6-h exposure to PFP73 ($P = 0.0034$), increased subsequent to PFP212 ($P < 0.0001$), and remained unchanged from DFP230 ($P = 0.0675$), compared with FA control animals (Fig. 6A). To qualitatively measure protein abundance, paraformaldehyde-fixed lung sections were stained against PCNA immunohistochemically. Basal PCNA positive cells, identified as cells containing dense nuclear staining, were abundant across both airway epithelium and the peribronchiolar interstitium (Fig. 6B). Abundance was diminished in PFP212 (Fig. 6C) and DFP230 (Fig. 6E) groups, but a plethora of PCNA positive proliferating cells were observed in the PFP73 group (Fig. 6D).

DISCUSSION

In this study, we investigated the effects of three different laboratory-generated particle exposures (DFP230, PFP73, PFP212) during lung development on architecture and lung mechanics. Each particle studied had a different effect on airway developmental indicators. Early-life exposure to PFP73 altered distal airway architecture and resulted in subtle changes in lung mechanics. Early-life exposure to PFP212 did not alter lung architecture but did alter lung mechanics in a manner suggestive of central airway changes. In contrast, early-life exposure to DFP230 did not alter lung architecture or mechanics. A single exposure to PFP73 decreased airway cell proliferation, while PFP230 increased it and 20% of particles did not. In general the particles that were highest in organic carbon deposition had the greatest effects on lung mechanics and airway architecture.

PFP73 exposure resulted in significant decreases in airway diameter and length, smaller branching angle both in proximal and distal airways, and smaller rotation angle in the middle generations. Since viscous resistance is inversely proportional to the fourth power of diameter in Poiseuille flow for a given volume flow rate (39, 40), changes in airway diameter observed here can seriously affect flow rates and pressure distributions associated with airway collapse during forced expiration (30). Theoretical prediction of overall resistance assuming Poiseuille flow (4, 18) showed that total resistance in the PFP73 group was larger than FA by 10% but the difference was not statistically significant ($P = 0.14$); PFTs also did not find significant differences in resistance (Table 3). Two factors could result in this observation: 1) shorter airway length offsets part of the resistance increase by smaller airway diameter and 2) changes in diameter and length in middle generations, where the resistance is highest, were small compared with distal and proximal region. Using a different, manual analysis method, airway size changes have also been observed by Plopper and colleagues in distal airways of Rhesus monkeys following postnatal exposure to another air pollutant, ozone (9). The automated geometry extraction methods used in the current study are a more powerful approach that measures differences in multiple airways in animals as small as young adult rats. This approach indicates decreases in airway size throughout the generations, providing a quantitative assessment of airway structural alterations (28, 29).

Despite the fact there were no significant airway architecture changes in the PFP212 group, a significant increase in airway resistance was observed after postnatal chronic exposure as well as an increase in cell proliferation following an acute exposure. The difference in airway resistance could be due to increased airway smooth muscle tone that would not be detectable in a fixed lung cast. Both airway smooth muscle abundance and airway tethering could be affected by the abundant cell proliferation that was detected within and around airways exposed to PFP212.

A striking feature illustrated in this study is that branching angles and rotation angles decreased in the PFP73 group. Remodeling in the airway tissue and changes in smooth muscle orientation could result in such angle changes. Branching angle will affect pressure drop in branching airways (23, 31, 37, 40) and rotation angle will be associated with optimal space filling of airways (26). Since the pressure drop in the complex branching architecture is dependent on variables such as diameter, length, airway size reduction ratio, and branching angle, more targeted studies will be required to elucidate the specific effects of airway angle changes on lung function.

The decrease seen in airway responsiveness in the PFP212 group is likely due to a change in baseline $R_s$; this baseline change increases the change in $R_s$ needed to produce a methacoline-induced 200% increase. While $R_s$ was not significantly affected by exposure to PFP73, I was significantly decreased, suggesting the observed changes in airway branching angle result in a decreased pressure gradient needed to initiate flow within the airway. In contrast, the significant increase in $H$ observed with exposure to DFP230 suggests an increase in the transpulmonary pressure needed to change lung volume. These observations are consistent with mild parenchymal remodeling.

In this study, DFP230 and PFP212 did not induce any significant alterations in the airway architecture while the
PFP73 group presented significant changes (Figs. 2–5). Particle deposition models in adult rat lungs (47) predict that a total deposition of OC in PFP73 and PFP212 will be over 2.5 times and two times that of DFP230, respectively, and total deposition of EC in PFP73 will be less than one-half of DFP230 or PFP212. Deposition of OC in PFP73 will be even higher postnatally because smaller airways will enhance diffusional deposition of these small particles. This implies that lung damage due to small particles may be caused by organic carbon mass rather than elemental carbon (54). This is consistent with other studies showing that particle size and surface characteristics are key determinants of toxicity for both fine and ultrafine particles (54, 55).

Our study suggests that an early decrease in normal cellular proliferation may be involved in the alterations in lung architecture in young rats exposed to PFP73 (Fig. 6). Reduced cell proliferation in developing animals exposed to particles is curious and the opposite of what is normally seen following exposure in adult animals, where lung cell proliferation usually increases following particle exposure (52, 55). However, the observation of reduced cell proliferation in developing animals is not unprecedented. A previous study by Pinkerton et al. (43) showed that exposure of neonatal rats for 3 days starting at 10 days of age to ultratine PM soot containing iron (target iron concentration was 100 μg/m³) also caused a decrease in cell proliferation in proximal alveoli that were within 300 μm of the terminal bronchiole-alveolar duct junction, but not in the terminal bronchiole. This exposure window is bracketed in the current study where exposure started at 7 days of age and continued exposures for 19 days. However, there are key differences between the two studies, such as particle concentration and composition. We did not measure changes in the terminal bronchiole, alveolar ducts, or alveoli as this is not compatible with the airway casting procedure. In another study, the early postnatal exposure to sidestream tobacco smoke impaired distal bronchiolar epithelial differentiation and proliferation in the postnatal rat lung (24). Even a transient decrease in normal cell proliferation could result in a decrement in overall lung growth later in life.

Another factor that is critical in interpreting these results is that neonatal lungs respond to toxicants differently than adult lungs. Previous studies of bioactivated lung toxicants, including some PAHs that are bound to particles, such as naphthalenes, have shown that neonates are much more susceptible to...
their toxic effects and show altered lung cell proliferation following exposure compared with adult animals exposed to the same dose (10, 11, 48, 49). The interaction between particle characteristics and the developing lung is critical for understanding the response of children to ambient air pollution. For example, several studies (6, 7, 38) suggest that children with asthma living near roadways with high diesel traffic have more asthma attacks. Diesel exhaust particles consist of elemental carbon and hundreds of compounds including polycyclic aromatic hydrocarbons and metals (50). Further investigation is warranted to define the effect of particulate matter air pollution on lung growth and development, specifically the role of various particulate components and particle sizes and their interaction with normal cell proliferation after both acute and chronic exposures.

These results support the epidemiologic observations that air pollution alters lung function growth in children. This study presents data demonstrating that postnatal exposure of rats to particulate air pollutants disrupts airway development, identifies particle properties that may be responsible for these observations, and indicates that early alterations in exposure-induced cell proliferation may be a critical event in the onset of persistent changes in airway architecture. Despite the tolerance of the obligate nose breathing adult Sprague-Dawley rat model to air pollutants, lung structure and mechanics were significantly changed when young animals were exposed to combustion-generated particles, even after a prolonged recovery period. Furthermore, the differential effect of exposure to DFP230, PFP212, and PFP73 on airway structure and mechanics illustrates the importance of particle size and composition. However, ambient particles are much more chemically complex than the ones we have employed in these studies and epidemiologic studies point to factors in the population, such as sex, genetics, and stress, that are also associated with lung function decrements. More studies are necessary to explore the full range of air pollutant and population characteristics that disrupt normal lung development.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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