Controversy persists regarding the validity of intratracheal instillation (IT) of particulate matter (PM) as a surrogate for inhalation exposure (IH) in rodents. Concerns center on dose, dose-rate, and distribution of material within the lung. Acute toxicity of a residual oil fly ash (ROFA) administered by IH was compared to those effects of a single IT bolus at an IH-equivalent dose. Male Sprague Dawley rats (60 days old) were exposed by nose-only IH to ~12 mg/m³ for 6 h. Inter-lobar dose distribution of ROFA, dissected immediately post exposure, was assayed by neutron activation. Vanadium and nickel were used as ROFA markers. IT administration of the IH-equivalent dose (110 µg) showed similar (<15%) interlobular distribution, with the exception of the inferior lobe dose (IT>IH~25%). Evaluation of airway hyperreactivity (AHR), bronchoalveolar lavage fluid (BALF) constituents, and histopathology was conducted at 24, 48, and 96 h post exposure. AHR in the IH group was minimally (p > 0.05) affected by treatment, but was significantly increased (~40%) at both 24 and 48 h post IT. Inflammation in both groups, as measured by alterations in BALF protein, lactate dehydrogenase and neutrophils, was virtually identical at all time points. Alveolitis and bronchial inflammation/epithelial hypertrophy were prominent 24 h following IT, but not apparent after IH. Conversely, alveolar hemorrhage, congestion, and airway exudate were pronounced at 48 h post-IH but not remarkable in the IT group. Thus, IT-ROFA mimicked IT in terms of lobar distribution and injury biomarkers over 96 h, while morphological alterations and AHR appeared to be more dependent on the method of administration.

**Key Words:** instillation; inhalation; ROFA; dosimetry; health effects.

Intratracheal instillation (IT) has long been used in toxicity testing to administer solid and liquid materials to the lungs of small laboratory mammals as screening method or as an alternative to inhalation (IH) exposure (reviewed by Driscoll et al., 2000). Its simplicity and the obviated need and cost for complex atmospheric generation and exposure systems have sustained its wide usage. Other advantages include the control of administered dose as well as the conservation of test material that may be of limited availability or expensive. Conversely, there are clear disadvantages of IT that often stimulate immediate criticism of studies that utilize the approach. Most notable among the concerns with IT is the issue of its non-physiologic means of delivery and instillate material dose-rate. The “bolus” delivery into the trachea is unlike the methodical cyclic deposition of the inhaled material over a fixed exposure period—usually on the order of several hours or days. The nose, which acts as an aerodynamic “filter” is an important deposition site for most particles, is bypassed, and the bolus administration of the instillate material may overwhelm mucociliary clearance mechanisms. Bolus administration of the IT material, although likely dispersed along the airways and into the deep lung by aspiration, might also exhibit a more heterogeneous intralobar distribution relative to the IH deposition pattern. This difference in distribution and the ability to clear particles could well affect the intensity and character of the response and lead to erroneous conclusions on the nature and potency of the material in question. Finally, IT administered materials are typically delivered at dose levels that far exceed those which would deposit under reasonable IH conditions and exposure durations, especially when linked to real-world exposure levels.

The utility and ease of use of the IT method has led to widespread use and has fueled the ongoing debate as to its relevance as a surrogate for IH. Driscoll et al. (2000), under the auspices of the Inhalation Specialty Section of the Society of Toxicology, conducted a thorough review and assessment of advantages and disadvantages of the IT method in rodent lung toxicity testing, and concluded that the method had merit in such testing, but with several significant caveats. The authors concluded that the method provides an expedient means of
administering compounds to the lung to conduct hazard identification (within the NAS paradigm—NRC, 1983) and does allow for comparative toxicity evaluation among test materials, especially when they are of limited availability. However, the authors noted that, in addition to the concerns discussed above, limitations exist on the interpretation of potential dose-related mechanisms and the confidence in long-term biologic outcomes since normal clearance processes can be overwhelmed and relevant microdosimetry with its associated toxicity might be altered. Nevertheless, there was consensus on the value of the method as a screening tool that could be used with the noted considerations and cautions.

Interestingly, despite many publications contending comparisons of IT and IH exposure outcomes, Driscoll and coauthors were impeded by the paucity of truly comparable IT and IH exposure-dose regimes. Many studies controlled for the administered material, animal species, etc., but for a variety of reasons the studies used multiple-day IH exposures or otherwise mismatched IT-IH exposure/dose concentrations or varied exposure durations (Brain et al., 1976; Hatch et al., 1981; Henderson et al., 1995; Prasad et al., 2000; Pritchard et al., 1985). Where attempts have been made to control for the doses delivered over narrow durations (Leong et al., 1998; Osier and Oberdörster, 1997), the inhalation methods employed intratracheal aerosol devices requiring anesthesia and/or ventilation thereby introducing other “non-physiologic” exposure attributes. Nevertheless, these studies collectively suggest that while the intensity of the IT instillation response is generally greater and more prolonged than that of the IH exposure, the qualitative aspects of the responses of the two methods are similar. Data from studies using lower, more relevant IH-IT doses suggest that the responses exhibit even better quantitative comparability and might offer utility in mechanistic studies to enhance risk assessment evaluations.

Recent interest in the health effects of ambient PM and engineered particles have raised interest in IT exposure methodologies. Standard IH exposures are often not practicable for ambient IH, and the availability of bulk samples ambient or engineered particles has raised interest in IT exposure and might offer utility in mechanistic studies to enhance risk considerations and cautions. Comparisons of IT and IH exposure outcomes, Driscoll and coauthors have raised interest in IT exposure and might offer utility in mechanistic studies to enhance risk.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Number/group</th>
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<td>0 h Post exposure</td>
<td>24 h Post exposure</td>
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<tr>
<td>Inhalation Air</td>
<td>N = 4</td>
<td>V, Ni Deposition/lobe</td>
<td>AHR, PATH, BAL</td>
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<tr>
<td>ROFA 12 mg/m³</td>
<td>N = 4</td>
<td>V, Ni Deposition/lobe</td>
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<tr>
<td>Instillation Saline</td>
<td>N = 6</td>
<td>V, Ni Deposition/lobe</td>
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<tr>
<td>ROFA 110 µg/rat</td>
<td>N = 6</td>
<td>V, Ni Deposition/lobe</td>
<td>AHR, PATH, BAL</td>
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MATERIALS AND METHODS

Animals. Sixty-day-old, male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were maintained in AAALAC approved facilities, housed in pairs within polycarbonate cages (25 cm × 15 cm × 50 cm) containing laboratory-grade pine shaving (North Eastern Products Corp., Warrensburg, NY). Facility rooms were maintained at constant temperature (22°C), humidity (50% RH) and 12 h light, 12 h dark illumination-cycle. Access to food (Rodent Chow 5001; Ralston Purina Laboratories, St. Louis, MO) and tap water was available ad libitum. Protocols were reviewed and approved by the NHEERL Institutional Animal Care and Use Committee prior to initiation of this study.

Particles. Residual oil fly ash (ROFA; Southern Research Institute, Birmingham, AL) was collected in 1979 at a Florida Power & Light plant that burned #6 grade residual oil containing 1% sulfur. Details on collection and composition have been reported elsewhere (Costa and Dreher, 1997; Dreher et al., 1997). Neither the composition of this ROFA nor its potency has altered over time. Elemental and sulfur analyses were performed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES: Perkin-Elmer Corp., Norwalk, CT: model P40). Inorganic and organic constituents of ROFA were also measured (Galbraith Laboratories, Inc., Knoxville, TN). The primary bioavailable metal constituents of this ROFA comprise V, Ni, and Fe, which because they exist in sulfate form, are readily water and weak acid soluble. The respective concentrations of these major metals in ROFA as determined by ICP-AES are 41.7 µg/mg, 37.5 µg/mg, and 23.3 µg/mg. Lung tissue levels for V and Ni following IH were used, as described below, to determine ROFA dosimetry in the lungs of exposed animals. Endotoxin content (ascertained because of its proinflammatory potential) was measured with the limulus amebocyte lysate assay with E. coli endotoxin as the control standard (Assoc. of Cape Cod, Inc., Woods Hole, MA). The MMAD and associated geometric SD (±g) as dry powder ROFA were determined using an optical particle counter (Thermal Systems, Inc., St. Paul, MN: model 3310A) were found to be −1.95 µm, ±g = 0.18 (Dreher et al., 1997).

Experimental design. Two separate experiments formed the basis of this study (Table 1): an inhalation (IH) exposure with rats exposed to 12 mg/m³ ROFA for 6 h in a nose-only exposure chamber, and an intratracheal-instillation (IT) exposure with rats receiving a predetermined dose of ROFA derived from
assay of the total (trachea and lung) inhaled dose, but administered instead in a single IT bolus. The IH lung-lobe distribution of ROFA was determined in a cohort of rats killed immediately post exposure. A cohort of animals IT dosed with this dose was also assayed immediately post exposure to compare the relative lobar dosimetry at that starting dose time point. At 24, 48, and 96 h post-IH or IT exposure, identical biological endpoints were evaluated in each experiment: airway hyperreactivity (AHR), cell and injury markers in bronchoalveolar lavage fluid (BALF), and pulmonary histopathology (24 and 48 h only).

Lung burden particle analysis. Dissected lobes and trachea from exposed rats were weighed and stored frozen in non-interfering cuvettes until neutron activation analysis (NAA). The NAA process consisted of 120 s irradiations at 1.5 × 10^{13} neutrons/cm^2 s followed by a monitored decay, after which each sample was counted for 200 s with Ortec 38% and 42% GeLi detectors coupled to an Ortec Omnigam-N Computerized Gamma Detection System (Dr. Jack Weaver; Department of Nuclear Engineering, College of Engineering, North Carolina State University, Raleigh, NC). Known reference analytical standards were employed: NIST SRM 1566A, NIST SRM 1084, NIST SRM 8505, and NIST SRM 1632-A—each achieving accuracy between 0.6–1.4% of their certified values. Reference standards of V and Ni were also employed.

Inhalation exposure. Administration of ROFA was conducted in awake, restrained rats using a dry dust aerosol generator (Ledbetter et al., 1998) in conjunction with a flow-by, nose-only exposure chamber. Briefly, the generator employed a continuously moving string to carry adherent particles upward from a dust reservoir past an air jet; aerosol production was accomplished using compressed air to dislodge particles from the string, discharging them into a 2 mCi 85Kr charge neutralizer/mixing chamber (Thermal Systems, Inc., St. Paul, MN). The aerosol then flowed vertically downward at a rate of 12 l/min into the 4 row, 24 port exposure chamber. Each exposed animal was restrained in a separate, conical, plastic tube (Lab Products, Inc., Seafood, DE). Chamber aerosol concentration was determined gravimetrically on samples collected approximately every hour from an unoccupied exposure port. Determination of aerosol-size distribution was performed at least once per exposure using a seven-stage cascade impactor (Intox Products, Albuquerque, NM). Chamber temperature, relative humidity and airflow were monitored continuously: a constant internal pressure, approximately 0.1 inch H2O, was maintained within the chamber at all times. Particle size as MMAD was measured during the exposure and did not differ significantly from that of the test runs with the same generating method and material (~1.95 μm, σg = 0.18).

Intratracheal instillation. Delivery of ROFA was performed using an endotracheal tube (Angiocath, 16 g: Becton-Dickinson Vascular Access, Sandy, UT) and fiberoptic laryngoscope (Costa et al., 1986) on rats anesthetized with halothane vapor (Aldrich Chemical Company, Milwaukee, WI). A 0.37 mg/ml suspension was prepared using sterile saline (Fujisawa USA, Deerfield, IL) then sonicated for 10 min; delivery of a 0.3 ml volume was accomplished using a syringe and catheterer (Angiocath, 18 g: Becton-Dickinson Vascular Access, Sandy, UT) inserted through the endotracheal tube. A dose response curve for IT ROFA (50 to 2500 μg) was conducted using total protein in bronchoalveolar lavage fluid (BALF—described below) as a marker of injury to ascertain the sensitivity and linearity of response at 24 h post treatment. In the comparison study, rats received a nominal dose of 110 μg ROFA IT as determined (using V and NI as tracers of ROFA deposition) from the combined lobar/tracheal dose of rats exposed by IH.

Bronchoprovocation testing. Airway hyperreactivity (AHR) using non-specific agonist bronchoprovocation was assessed on each rat to evaluate airway function changes. Each rat was anesthetized with urethane, 1 g/kg, ip. (Sigma Chemical Co., St. Louis, MO), surgically fitted with an indwelling jugular vein catheter (PE-50: Becton Dickinson & Co., Sparks, MD), and intubated with a 12 gauge endotracheal tube molded from polyolefin, heat-shrink tubing (Digi-Key, Beef River Falls, MN). The rat was then placed supine in a constant-volume, whole-body plethysmograph and paralyzed with succinylcholine, 2.5 mg/kg, im. (Glaxo-Smith-Kline, Research Triangle Park, NC) and established with a constant-volume ventilator (Harvard Apparatus, South Natick, MA: model 683). Plethysmograph pressure was monitored by an isothermal-referenced pressure transducer (Validyne Engineering Corp., Northridge, CA: model MP 45-4) to derive tidal volume and time-differentiated flow signals. Airway pressure (Pao) in the endotracheal tube was also monitored (Electromedics, Inc., Englewood, CA: model MS20). The direct signals were digitized by an analog-to-digital converter every 2.5 msec with phasing inequalities between them corrected electronically using computer software. The ventilator was adjusted to maintain a respiratory rate of 90 breaths/minute and a tidal volume of 7.5 ml/kg for each rat to sustain arterial blood pH, pO₂, and pCO₂ homeostasis within normal ranges (Lehmann et al., 1997). Resistance and compliance measurements were computed from at isoflow and isovolume values of the respective tracings. The saline-filled, jugular-vein catheter was connected to a peristaltic pump (Isomatic SA, Zurich, Switzerland: model IP-12) through a portal in the plethysmograph face plate. Following the initial baseline measurement of airway pressure during a 1 min interval without infusion and a 1 min interval with saline infusion at 0.04 ml/min, the rats were continuously infused with acetylcholine (Ach; Sigma Chemical Co., St. Louis, MO) using a dilution adjusted for body weight, 900 g/ml/kg. Flow rate of the peristaltic pump doubled every 2 min, from 0.04 ml/min to 0.64 ml/min, resulting in a total delivered dose of 2.23 mg Ach in a total volume of 2.5 ml saline. A pentium based workstation, using software developed with the LABVIEW graphical programming language (National Instrument Corp., Austin, TX), was constructed to operate the electromechanical components of the system, as well as collect, compute, and store data.

Bronchoalveolar lavage fluid (BALF). Following completion of AHR, the rats were euthanized by exsanguination from the descending aorta. Saline, 28 ml/kg, was injected into the trachea of each rat; lungs were lavaged three times with a single volume. Total cell counts were performed using a Coulter counter (Coulter Electronics, Hialeah, FL: model ZBI). Differential cell counts were performed on cytocentrifuge preparations (Shandon, Pittsburg, PA: Cytospin Model II), stained with Diff-Quik (American Scientific Co., Sewickley, PA); two hundred cells per slide were enumerated. Biochemical analyses were performed on supernate following centrifugation of BALF at 500 g for 10 min at 4°C using assays modified for use on a centrifugal spectrophotometer (Hoffman-La Roche, Branchburg, NJ: Cobas Fara II). All samples were analyzed for total protein (TPR; Comassie plus reagent: Pierce and Co., Rockford, IL; bovine serum albumin standards: Sigma Scientific Co., St. Louis, MO) and lactate dehydrogenase (LDH; Assay kit 228: Sigma Chemical Co., St. Louis, MO). TPR was used as an indicator of plasma infiltration or edema marking blood-air barrier damage and LDH was used as a nominal marker of lung and airway cell damage.

Pathological evaluation. Left lungs from subsets of control and exposed rats of both the IH (n = 4/timepoint) and IT (n = 6/timepoint) experiments were infused via the trachea with filtered, 4% parafomraldehyde, prepared in phosphate buffered saline, pH 7.2. The inflation volume, 11.2 ml/kg, was injected into the trachea of each rat; lungs were lavaged three times with a single volume. Inflated lungs were stored at 4°C until embedded in paraffin. Mid-sagittal sections, 4 μm thick, were stained with hematoxylin/eosin then evaluated by a veterinary pathologist using light microscopy (Experimental Pathology Laboratories, Durham, NC). The degree of lung injury was estimated by assigning a semi-quantitative score (0–4) for separate categories representing degenerative, proliferative, and inflammatory alterations, based on incidence and severity and location (bronchial and alveolar). The classification categories included: alveolitis, edema, hemorrhage, epithelial desquamation, peribronchial, and perivascular infiltration of both mononuclear cells and granulocytes.

Statistical analysis. Data were analyzed using two-way analysis of variance (ANOVA) to ascertain the effect of time and treatment on the biological endpoints measured. Effects were considered significant when p ≤ 0.05. Pairwise comparisons were performed as subtests of the overall ANOVA model. The level of significance associated with multiple comparisons was adjusted with a modified Bonferroni correction.
RESULTS

ROFA Distribution

The distribution of ROFA to each of the five lung lobes and trachea of three ROFA rats was determined from tissues harvested immediately post-IH exposure. Actual ROFA dosages were computed means derived from V and Ni tissue levels each corrected for tissue background levels for these metals. Each lung lobe (and trachea) was assessed for Ni and V content by neutron activation. After adjusting for the ROFA/metal ratios to compute the ROFA dose to each lung compartment, a total lung dose was represented by this summation. The total IH dose after 6 h was computed to be nominally 110 µg with a CV of 15%. This 110 µg dose was used for the IT studies, including the assessment of ROFA lobar distribution as well as the biologic responses over the ensuing 96 h. Six cohort rats exposed IT were similarly sampled immediately post IT. Figure 1A represents the distribution by lung lobe and trachea for the IH and IT exposures. Plotted along with these data are the estimated lobar doses based on previous IT studies using 7Be-labeled carbon PM (Costa et al., 1986). This estimation is provided to demonstrate the reproducibility of the IT methodology when it is implemented in a standardized manner. It is clear from this representation that the distribution by lobe between the two methods of exposure does not differ significantly. Moreover, when compared to historic data using a different particle (~1.5 µm aggregates of primary carbon particles; CMD: 0.027 µm), the distribution was very reproducible. Using the lung lobe weights taken at sampling, the specific lung tissue ROFA dose (µg ROFA/g lung tissue) could be computed and is represented in Figure 1B. The similarity of the tissue dose (as µg/g lung tissue) across all lobes suggests that there was no major disparity in lobe to lobe distribution with IT procedure—a finding also consistent with our earlier study (Costa et al., 1986). By inference from the data in Figure 1A, the same lobe to lobe consistency of dose should also be true.

Bronchoprovocation

Groups of six rats were evaluated at each time point after IH and IT ROFA exposure for airway responsiveness to non-specific agonist challenge. The agonist challenge was to iv Ach, which is known to induce bronchoconstriction in a dose dependent manner reflecting generalized airway responsiveness to nonspecific stimulation. When the airway is damaged, inflamed, or otherwise remodeled due to chronic disease (e.g., asthma), it exhibits an exaggerated responsiveness to the iv Ach challenge. We have shown previously that larger IT doses of ROFA induce airway hyperreactivity (AHR) as assessed by this methodology (Gavett et al., 1997). Using ED_{150} values for Ach (Fig. 2), the IT-ROFA doubled the airway responsiveness at 24 h, with little change through 96 h. On the other hand, the IH-ROFA exposure did not show a clear impact at 24 h as with the IT exposure, but rather displayed a progressive trend of increasing reactivity over the 96 h study period. However, AHR for the IH group never reached statistical significance.

Pulmonary Injury and Inflammation

The impact of IH and IT ROFA on lung inflammation end points at 24 through 96 h post exposure was quantitatively similar. Figures 3A and 3B represent the temporal BALF protein and LDH responses to IH and IT ROFA showing progressive worsening of these indices of injury between 24 to 96 h post exposure. However, and more importantly to the point of the study, there were no significant differences in these indices of pulmonary injury/inflammation between the IH and IT exposures at 24, 48, and 96 h post-exposure. In temporal contrast, inflammation, as represented by infiltrated BALF neutrophils (Fig. 3C), peaked at 24 h and decreased thereafter through 96 h post IH and IT exposures. The BALF neutrophil...
counts following 110 μg IT-ROFA exposure were only slightly, but significantly greater than the IH counts at most time points, as were the saline IT versus the air IH counts. However, if adjusted for the control values (as % increase over the corresponding control or as % change) the responses were essentially identical over 24 to 96 h post-exposure.

To ensure the validity and reproducibility of the BALF response to ROFA, a dose-response curve for lavageable protein was generated for IT doses 50–2500 μg at 24 h post exposure. Figure 4 represents this near-linear (r² = 0.98) over the dose-range assessed.

**Lung Pathology**

Semi-quantitative histopathological assessment of left lung sections was conducted in a blind fashion relative to exposure. A number of lesions were noted in the lungs of all groups. The least involved lungs showed some macrophage accumulation in the air spaces, but exposed animals had varying degrees alveolitis, predominated by acute neutrophil followed by delayed mononuclear cell infiltration. Vascular congestion and perivascular edema was also evident. Airway injury was noted by epithelial desquamation with intact or degenerating cells free in the lumen of small bronchi along with proteinaceous exudates and occasional evidence of alveolar hemorrhage. Bronchial epithelial hypertrophy appeared by 48 h as pseudostratified and regenerating epithelia along the airway surface. The IT groups (n = 6/group) showed more significant macrophage alveolitis as well as more substantial bronchial inflammatory cell and fibrinous fluid infiltrate. Congestion of small airways and alveolar hemorrhage was seemingly greater in the IH groups (n = 4/group). In general terms, the intensity of pathology appeared greater at 24 h for the IT group, but was somewhat greater at 48 h for the IH group (Figs. 5A and 5B).

Average lesion scores for both the IT and IH groups showed a similar pattern of airway/alveolar damage. There was a trend toward more damage at 24 h post IT-ROFA than with the IH group. This scoring is consistent with the overall pathology descriptive accounts, but does not reflect the subjective impression of airway-lesion dominance in the IT group. What is more striking is the evidence of lesion resolution in the IT group at
48 h and the clear worsening or progression of bronchial and alveolar lesions at that same time point for the IH group (Fig. 5C). Pathologic evaluations were not conducted at 96 h.

DISCUSSION

The environmental and occupational health communities have an expanding need for relevant assessments of the impacts of inhaled substances on the cardiopulmonary system. When human data are not available, data derived from inhalation exposure studies using animal models remains the “gold standard” for determining the potential toxicity of inhalants and to explore the underlying mechanisms that support risk assessments. Of particular interest is the concern regarding the health effects of ambient PM that, in recent years, have come to the forefront of environmental toxicology. The physical and compositional complexity of ambient PM and the difficulties conducting the spectrum of inhalation studies needed to address specific questions regarding suspect hazardous components or attributes and associated mechanisms of action have pressed for alternative methods of pulmonary exposure. Similar issues exist with regard to newly engineered nanoparticles which have limited availability, are expensive, or are difficult to dispense for inhalation study (Oberdörster et al., 2005a,b; Warheit et al., 2004). In the unique circumstance of nanoparticles, the concerns that have arisen that IT methods would distort particle-size associated effects, have been somewhat allayed by studies with ultrafine particles. The studies of Osier and Oberdörster (1997) made such a direct comparison using agglomerates of 20 nm TiO2 particles as compared to unitary
particles of 250 nm and showed that the heightened potency of nanometer particles was not lost when administered IT.

Intratracheal instillation has a long history in toxicology, having its origin in occupational health studies of mineral dusts such as silica and asbestos, where considerable doses of these materials have been instilled to assess the pathophysiology of ensuing lung lesions and disease (reviewed by Driscoll et al., 2000). Even today, these high dose studies are used routinely to explore mechanisms of disease and potential interventions (Taylor et al., 2003; Warheit et al., 2005). However, there is considerable skepticism, more in the environmental than in the clinical community, on the validity of the IT method, even at relatively low lung doses. The concerns range from the non-physiologic means of exposure and dose-rate, as well as potentially overwhelmed clearance processes which may result in prolonged residence times in the lung for the particulate material to interact with airway and alveolar cells. Many studies have been published over the years attempting to address IT-IH issues, most being limited by IT-IH lung-dose inequities, incomparable exposure durations or scenarios, or other artifacts of the study design (reviewed by Driscoll et al., 2000). On the one hand, the studies of Henderson et al. (1995) which matched the IT doses of TiO2 and SiO2 to lung burdens after one week of exposure found that the 24 week pathology (and interim BALF indicators in the case of SiO2) that compared reasonably well between IT and IH over extended response times from 1 to 24 weeks post exposure. On the other hand, short-term responses were tracked after very high IH exposures via an intratracheal port were used to achieve measurable lung burdens that could be used for IT. These studies involved ventilated animals that could revive and be followed up to a week post exposure. Again, there appeared to be generally good agreement between the methods although the IT response somewhat exceeded the IH. The present study was designed to go beyond these studies to compare directly IT administered particulate material at a low lung dose determined from an acute nose-only IH exposure. Lung dose distribution and resultant clinically relevant biological outcomes were compared for up to four days post exposure.

The emission PM used in the study, ROFA, is one of many fly ashes that have been used as surrogate PM in lung toxicity and mechanism studies (Dreher et al., 1997; Gavett et al., 1997; Kodavanti et al., 1998, 2002). As the particular ROFA used in this study has been well characterized in our laboratory (physicochemically and biologically), we chose to use it to ensure controlled exposures and in anticipation of clearly measurable biological effects. We selected four main issues to investigate: lung distribution, airway physiology, BALF indices of injury/inflammation, and airway/lung histopathology.

Initially, we determined the lobe to lobe ROFA particle distribution after 6 h exposure to 12 mg/m3 ROFA aerosol using V and Ni as intrinsic tracers (based on neutron activation and decay). While the two metals showed a slight difference in residence time (Ni>V) based on measurements immediately after exposure, the ROFA dose to each lung lobe was computed from their averaged tissue concentrations (the coefficient of distribution was ~15%). A cohort of rats instilled with the assayed total lung dose of 110 μg underwent similar lobe by lobe analysis for ROFA and showed a similar Ni>V pattern. The lung burdens were not assayed at the 24–96 h assay points, so the significance of any difference in Ni:V clearance is unknown. It has been shown, however, that V may clear somewhat faster from the lung than Ni (Edel and Sabbini, 1988; Pierce et al., 1996), but differences in the temporal clearance between inhaled and instilled Ni do not appear to be significant (Hirano et al., 1994). Nevertheless as can be seen clearly in Figure 1A, the relative lobe distribution of ROFA was virtually identical with both IH and IT immediately post exposure, and the pattern agreed well with previously reported model PM distribution (Costa et al., 1986). Moreover, when normalized for lobe mass, the tissue-dose (mg/g) was very similar lobe to lobe. A similar homogeneity from lobe to lobe in dose/g tissue was reported recently by Cassee et al. (2002) for IH CdCl2 aerosols of various sizes. Unpublished data from our laboratory (Wichers, in preparation) using a much less soluble ROFA administered by acute IH also showed this same distribution pattern, suggesting that neither solubility nor particle size is highly determinant of the lobe to lobe pattern—although their impacts on absolute doses are evident.

While intriguing, this finding of lobe to lobe consistency when weight-adjusted should not be construed to imply that the within lobe distribution (at the cellular level) was also constant and similar from IH to IT. This study did not address the issue of within lobe deposition, although the pathology differences may reflect differential microdeposition patterns. Indeed, several studies have shown that IT often results in end-airway accumulation of the instilled material and, not surprisingly, the site of primary lesion (Dorries and Valberg, 1992; Henderson et al., 1995; Osier and Oberdörster, 1997; Vogel et al., 1996). Osier and Oberdörster (1997) reported semi-quantitative evidence that inhaled particles reached the alveoli in greater number. On the other hand, some studies have not found substantial differences between the methods (Muller et al., 1989; Warheit et al., 1991). Instilled fibrous materials appear to pose the greatest end-airway accumulations relative to inhalation (McConnell, 1995; Morgan, 1995). In contrast to the 110 μg instilled herein (~400μg/kg), the doses in these studies ranged from 5 to 500 times greater. Those studies at the lower end of this range suggested (though it was not emphasized in those studies) of more even distribution than with the IT administration. Yet, even at IT doses in the 2–4 mg/rat range, the IT distribution when conducted by experienced investigators consistently follows the lobar mass ratio as was observed here (personal observation). It appears that the evenness of the lobe mass-based distribution reflects the lobe volume proportionality to lobe mass. With aspiration of the material instilled at the carina would distribute in proportion to volume of each lung lobe. An analogous pattern of dispersion is seen with
particle deposition during normal tidal inhalation in the rodent (Cassee et al., 2002; Raabe et al., 1975; this study).

As the objective of the study was to compare the pulmonary responses to the particle toxicant, ROFA, when administered by IT or IH at equivalent lung deposited dose levels, the focus of the assessment was on the biological outcomes over the period of analysis. The impetus for assessing airway function arose from the current concerns regarding the health impacts of ambient PM, most notably on asthmatics. Many field studies have impugned PM with increased inhaler use, hospitalization, and other untoward airway associated effects (AQCD for PM, 2004). Likewise, our studies have shown airway effects with various PM, including surrogates and ambient material (Dick et al., 2003; Dye et al., 1999; Gavett et al., 1997). We also anticipated that the immediate effect of IT versus IH-ROFA would likely be most evident in airway function. Hence the increased airway reactivity to Ach at 24 h post treatment was not surprising. We had observed a similar response previously with another ROFA-IT study at higher doses (Gavett et al., 1997). However, the sustained effect at 96 h was somewhat surprising given the relatively low dose. Previous studies had showed a similar sustained effect at that time point, but at doses many times greater. Interestingly, the IH-ROFA which did not have the 24 h effect showed a definitive trend toward increased reactivity with time through 96 h. This may relate to the toxicity of ROFA to airway cells that simply was expressed more slowly when the particle deposited by inhalation. The temporal difference in response might also relate to the relative dosimetry to the tracheobronchial region of the two exposure methods. The deposition fraction to the airways for the IH-ROFA is estimated as ~6% (Asgharian et al., 2001), which compares to a deposition fraction to the trachea of ~15% (data not shown) with the IT-ROFA study. However, it remains that the qualitative nature of the response in the context of hazard identification, was similar with both exposure methods.

We have found with other ROFA studies that airway responsiveness recovers in about 7–10 days (at higher doses—unpublished data). Hence, it is likely that the AHR in the IH study would also fully recover in this time frame.

The BALF data, on the other hand, indicated little difference between IT and IH at any of the post exposure time-points. The BALF protein used as an index of blood-air barrier integrity showed a progressive increase of similar magnitude from 24 to 48 h and then a plateau through 96 h. These changes were paralleled by the BALF LDH values, indicative of cell damage and perhaps some blood plasma leakage into the airspace (LDH isoforms were not discriminated). Likewise, inflammation in the form of lavageable neutrophils increased immediately with both exposures and slowly waned with time. The slight difference between the exposure methods (IT>IH) was reflected in the saline controls as well. When this subtle but real control IT-saline response was subtracted from the ROFA response, the inflammatory responses were also virtually identical for the two procedures.

The pathology findings were generally reflective of the BALF data indicative of epithelial damage and inflammation. The “acuteness” of the IT response was somewhat evident as was the apparent intensity of the airway cell damage and fluid infiltrates. Thus, by 48 h the IT lesions showed apparent epithelial proliferation and signs of repair. The distribution of injury was generally similar in both groups, but there was suggestion that the alveolitis was somewhat stronger in the inhalation group, perhaps due to more particles penetrating to that depth. These latter findings have been reported by others, especially for fibers (Dorries and Valberg, 1992; Henderson et al., 1995; McConnell, 1995; Morgan, 1995; Osier and Oberdörster, 1997; Vogel et al., 1996). The scoring of the lesions did not correlate well with the qualitative attributes of the bronchial-alveolar impacts. In fact, by comparison, the more intense IT 24 h lesions in the airways showed some recovery by 48 h, when the IH rats showed peaking injury. This difference is intriguing in light the equivalent total dosimetry and the general belief that the “bolus” exposure with IT would have more dramatic long-term consequences because of the more intensive acute injury. When combined with the airway reactivity data, it suggests that while the nature of the various functional and tissue lesions may be qualitatively similar, the actual quantitative differences in response may not be clear-cut, but may have a temporal element that plays into the response scenario—and any subsequent risk calculation.

In conclusion, this study supports the argument that the use of the IT methodology in lieu of IH can provide relevant information regarding the basic toxicity of an inhalable material. The distribution of regional doses (lobe to lobe) was virtually identical between IT and IH exposure regimes, although there was evidence of within lobar differences—i.e., small airway to alveoli. Thus, site specific differences (e.g., airway responses) might be acutely heightened. The nature of the biological responses, and to a large extent the magnitude of these responses, at least in the context of parameters assessed herein, seem to be quite similar. Indeed, there were temporal differences that cannot be ignored especially regionally. However, they reflected more the time to response than the nature of the response itself. Nevertheless, it is reassuring that the BALF fluid response measures were so similar in magnitude as well as temporally. What has not been addressed in detail is the influence of dose. The dose response curve for lavageable protein that was generated at the outset of the study to validate the responsiveness of the IT component of the study was essentially linear. While this may not reflect on other end points and parameters, it does suggest that there is some homogeneity in the response to ROFA over a large IT dose range and that studies across this range provide relevant information in the assessment of particles. Unquestionably, the lowest and most relevant IT dose relative to reasonable IH exposure concentrations will yield the most relevant data. Nevertheless, it is clear that judicious use of IT as a means of administering particulate toxicants to the lung of rodents can be
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REFERENCES

Air Quality Criteria Document (AQCD) for Particulate Matter (PM) (October 2004) NCEA/EPA – website access @ http://cfpub.epa.gov/ncea/cfm/partmatt.cfm.


