A Pilot Investigation of the Relative Toxicity of Indoor and Outdoor Fine Particles: In Vitro Effects of Endotoxin and Other Particulate Properties

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In this study we assessed the in vitro toxicity of 14 paired indoor and outdoor PM2.5 samples (particulate matter ≤2.5 µm in aerodynamic diameter) collected in 9 Boston-area homes. Samples were collected as part of a large indoor particle characterization study that included the simultaneous measurement of indoor and outdoor PM2.5, particle size distributions, and compositional data (e.g., elemental/organic carbon, endotoxin, etc.). Bioassays were conducted using rat alveolar macrophages (AMs), and tumor necrosis factor (TNF) was measured to assess particle-induced proinflammatory responses. Additional experiments were also conducted in which AMs were primed with lipopolysaccharides (LPS) to simulate preexisting pulmonary inflammation such as that which might exist in sick and elderly individuals. Significant TNF production above that of negative controls was observed for AMs exposed to either indoor or outdoor PM2.5. TNF releases were further amplified for primed AMs, suggesting that preexisting inflammation can potentially exacerbate the toxicity of not only outdoor PM2.5 (as shown by previous studies) but also indoor PM2.5. In addition, indoor particle TNF production was found to be significantly higher than outdoor particle TNF production in unprimed AMs, both before and after normalization for endotoxin concentrations. Our results suggest that indoor-generated particles may be more bioactive than ambient particles. Endotoxin was demonstrated to mediate proinflammatory responses for both indoor and outdoor PM2.5, but study findings suggest the presence of other proinflammatory components of fine particles, particularly for indoor-generated particles. Given these study findings and the fact that people spend 85–90% of their time indoors, future studies are needed to address the toxicity of indoor particles. Key words: alveolar macrophage, cytokines, endotoxin, fine particles, indoor air pollution, PM2.5, toxicity, tumor necrosis factor. Environ Health Perspect 109:1019–1026 (2001). [Online 26 September 2001] http://ehpnet1.niehs.nih.gov/docs/2001/109p1019-1026long/abstract.html

Given the fact that people spend 85–90% of their time indoors (1), it is widely recognized that a significant portion of total personal exposures to particulate matter (PM) occurs in indoor environments. Indoor particles are composed of both ambient particles, which infiltrate indoors, and nonambient particles, which are generated indoors during the daily activities of home occupants. In a previous paper (2), we demonstrated that indoor fine particle concentrations in nine Boston-area study homes were significantly elevated during cooking, cleaning, and other general indoor activities involving combustion (e.g., burning candles) or physical movement (e.g., walking). Indoor source events were typically of short duration, but many were of very high intensity, capable of raising hourly concentrations of PM2.5 (particulate matter ≤2.5 µm in aerodynamic diameter) by tens to hundreds of micrograms per cubic meter. Furthermore, this and other studies have shown that indoor particle events can substantially modify the size distribution and composition of indoor particles (2–7).

Exposures to indoor-generated particles may be highly relevant to public health because of the high frequency of exposure to large short-term events. In fact, concern over the health significance of exposures to peak short-term concentrations has grown due to the findings of several recent studies that short-term ambient PM events are associated with acute health outcomes (8–13). Due to differences in size distributions and composition, it is possible that indoor-generated particles may be more or less toxic than ambient particles.

However, given the U.S. Environmental Protection Agency’s (U.S. EPA) mandate to regulate ambient air pollution, epidemiologic and toxicologic studies have traditionally addressed only the health impacts of ambient particles. Over 150 epidemiologic studies have reported significant associations between ambient PM levels and excess mortality and morbidity (14). Among the adverse health outcomes that have been most strongly linked to ambient PM exposures are cardiopulmonary mortality, symptoms of respiratory and cardiovascular disease, and impaired lung function. Toxicologic studies are ongoing to determine the causal agents and underlying mechanisms for ambient PM health effects (15,16).

Due to their low cost and sensitivity, in vitro toxicity tests are beginning to be used more widely as exploratory tools in PM toxicologic studies. In vitro bioassays have been more extensively used to investigate the toxicologic properties of homogeneous particle mixtures including residual oil fuel ash, urban air particles (UAP), inert titanium dioxide, elemental carbon, and diesel particles (17–25). Only very recently have studies reported bioassay data for ambient PM1.5 and PM10 samples. These studies have demonstrated a variety of biological responses for alveolar macrophages, blood monocytes, and respiratory epithelial cells including cytotoxicity, particle phagocytosis, oxidant production, and production of inflammatory mediators (21–23,26–28).

Specifically, these studies have provided evidence that particle-bound endotoxin and trace metals contribute to the observed biological activity of ambient PM samples.

Despite the public health implications of indoor particle exposures, only one of these studies reported bioassay findings for indoor particles (26). In this study, we used in vitro bioassays to investigate the relative toxicity of indoor and outdoor PM2.5 that was collected from nine Boston-area homes as part of a large indoor particle characterization study. Similar to previous studies (23,29), the bioassays measured the tumor necrosis factor (TNF) released by rat alveolar macrophages (AMs) after exposures to indoor and outdoor particles. We chose TNF as the measurement end point because it is a potent proinflammatory mediator in the lung that has been shown to play a crucial role in the recruitment and activation of numerous inflammatory cells (30). To simulate preexisting pulmonary inflammation such as that which...
might exist in sick and elderly individuals, we also conducted bioassays using macrophages that were primed with lipopolysaccharides before the particle exposures.

### Materials and Methods

**Study design.** As described previously, we sampled nine nonsmoking Boston-area homes for 1 or 2 week-long periods during spring–summer and fall–winter 1998 (2). All homes were located within 30 miles of downtown Boston in suburban neighborhoods. Study homes were typical of homes in New England, a region in the United States with four distinct seasons including cold winters and warm summers. Windows and doors were predominately kept closed for the winter months as well as the majority of the spring and fall sampling periods. During the winter months, five of the nine homes were heated with oil, whereas the remaining four had natural gas heating systems. Similarly, five homes had radiant heat and four had forced-air heat. During the summer months, home occupants typically opened windows and doors to promote air circulation. The major exception was Home FOX1, which relied upon a central air-conditioning system during the summer months, including its July sampling event.

Five of the nine study homes were sampled during each of two seasons. All homes were sampled a minimum of 6 consecutive days on each sampling occasion, with most homes sampled for at least 7 days and several for longer periods. Table 1 summarizes the locations, sampling dates, and sampling duration for each study home.

**Toxicity sample collection.** Harvard Impactors (HI; Air Diagnostics and Engineering, Inc., Harrison, ME) were used to collect indoor and outdoor PM$_{2.5}$ samples for the bioassays. To obtain a sufficient mass of particles, these samplers were operated continuously for the duration of sampling at each home. Hence, one indoor and one outdoor sample were collected during each sampling period for a total of 14 indoor and 14 outdoor samples.

PM$_{2.5}$ HIs were operated at a flow rate of 10 L/min according to previously documented specifications (31,32). Flow rates were measured every 12 hr using calibrated rotometers, and flows were adjusted if they had changed by more than ±5%. Samples were collected on preweighed 37-mm Teflon filters (Teflo; Gelman Sciences, Ann Arbor, MI). All filters were on- and off-weighted twice using a Mettler MT-5 Microbalance (Mettler Toledo International, Inc., Greifensee, Switzerland) in a temperature- and relative humidity-controlled weighing room after at least 48 hr equilibration time. These weights were used to determine filter loadings (Table 1).

**Sample preparation and TNF bioassay.** Detailed laboratory methods describing sample preparation and the TNF bioassay have been previously reported (21–23,28,33). Briefly, we cut filters into tiny pieces and placed them in a sterile, endotoxin-free saline solution for sonication. The filter pieces were removed after the sonication procedure, and an aliquot of the suspension was dried on a preweighed Teflo filter so that the mass recovery from the filters could be calculated (Table 1). Average percent recoveries were 59 ± 6% (range 22–88%) and 69 ± 7% (range 20–99%) for indoor and outdoor samples, respectively. Neither recoveries nor bioassay results are reported for the Home MAN1 samples; these samples were used in an earlier set of bioassays, so very little sample remained. UAP standard reference material 1649 (National Bureau of Standards, Washington, DC), which consists of total suspended particulates collected in the 1970s in Washington, D.C., was selected as a positive control. We dissolved UAP in saline solution at 10 mg/mL. All particle suspensions were kept frozen (−20°C) until use.

We harvested rat alveolar macrophages (AMs) from two female CD rats (250–300 g body weight, virus antibody free, Harlan Sprague Dawley, Inc., Indianapolis, IN) by bronchoalveolar lavage (BAL) using a phosphate-buffered saline (PBS) solution. After centrifugation, BAL cells were resuspended at 1 × 10$^6$ cells/mL in an assay buffer solution consisting of RPMI-1640 media (BioWhittaker, Walkersville, MD) supplemented with 1% fetal bovine serum (FBS), 0.1% balanced salt solution, penicillin, streptomycin, and 1-glutamine (RPMI 1%).

For the second set of priming experiments, AMs were initially treated with a 200 ng/mL concentration of UAP (positive controls; n = 4); with UAP (positive controls; n = 4); and with indoor and outdoor particles (PM$_{2.5}$ samples; n = 52). Bioassays were repeated using primed AMs. We did not perform the TNF bioassay for either sample from Home MAN1 due to the extremely low recoveries. Briefly, we first dispensed 80 μL of assay buffer into each well. Next, we added 80 μL of either concentrated particle suspension (either indoor/outdoor PM$_{2.5}$ or UAP) or assay buffer (negative controls) to each test well; this was followed by 80 μL of cell suspension. Aliquots of each particle suspension were previously thawed, probe sonicated, and diluted so that a standard exposure concentration of 100 μg/mL was attained for each well. The plates were then incubated for 20 hr in a humidified incubator at 37°C with 5% CO$_2$. Upon completion, the well contents were placed on ice; an aliquot of supernatant was then removed and stored frozen for use in the TNF bioassay.

**Table 1.** Sampling locations and dates, and collected and recovered PM$_{2.5}$.

| Home ID | Home location          | Season  | Starting date | Sampling duration (days) | Collected mass (µg) | Recovery (%) |
|---------|------------------------|---------|---------------|--------------------------|---------------------|--------------|
| MAN1    | Manchester-by-the-Sea  | Winter  | 13 Feb 1998   | 7                        | 992.5               | 249.5        | –            | –            |
| NEW1    | Newton                 | Spring  | 26 Mar 1998   | 9                        | 1397.5              | 1,457        | 22           | 39           |
| WEL1    | Wellesley              | Spring  | 29 Apr 1998   | 11                       | 2,420               | 1,568        | 68           | 20           |
| SWP1    | Swampscott             | Summer  | 28 May 1998   | 8                        | 1,583               | 819.5        | 51           | 82           |
| BOX1    | Boston                 | Summer  | 9 Jun 1998    | 9                        | 1,074               | 1,211        | 35           | 41           |
| NEW2    | Newton                 | Summer  | 20 Jun 1998   | 6                        | 2,100               | 2,135.5      | 41           | 89           |
| FOX1    | Foxboro                | Winter  | 7 Jul 1998    | 9                        | 679.5               | 861          | 88           | 74           |
| WEL2    | Wellesley              | Winter  | 5 Nov 1998    | 7                        | 812.5               | 819.5        | 62           | 62           |
| SWP2    | Swampscott             | Winter  | 13 Nov 1998   | 7                        | 1,164 ± 56          | 1,098 ± 128   | 59 ± 5.5     | 69 ± 6.9     |

References:

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We conducted the TNF bioassay using a recently published microplate assay (33). This microplate assay uses a fluorescence-based quantification technique to assess TNF-induced cell death in the TNF-sensitive WEHI 164 clone 13 tumor cell line. Cell cultures were either dosed with AM supernatants or TNF standards (recombinant rat TNF; R&D Systems, Minneapolis, MN). We used a CytoFluor fluorescence plate reader (PerSeptive Biosystems, Inc., Framingham, MA) to measure propidium iodide fluorescence.

Endotoxin assay. Because other studies have shown that endotoxin is a potent stimulant of cytokine production (17,23,25,28), we also measured indoor as well as outdoor endotoxin concentrations for particle suspensions. Endotoxin was measured by *Limulus* assay (chromogenic *Limulus* amebocyte lysate kit; BioWhittaker) according to the manufacturer’s instructions. Endotoxin concentrations are reported as endotoxin units (EU) per milligram of particles where 10 EU is equivalent to 1 ng of reference standard endotoxin. Endotoxin was measured for both whole particle suspensions and supernatants after centrifugation, but we report only data for the whole particle suspensions. Whole particle suspensions had significantly higher endotoxin concentrations than supernatants (2.62 ± 0.67 EU/mg versus 0.60 ± 0.08 EU/mg). This finding, which suggests that endotoxin predominantly exists in a particle-bound form, is similar to that of a previous study of concentrations on average were greater than outdoor measurements were made at 15, 35, and 55 min after each hour. According to previously described methods (2), fine particle SMPS and APS data were converted to volume concentrations (cubic micrometers per cubic centimeter) for three particle size ranges: 0.02–0.1 μm (PV0.02–0.1), 0.1–0.5 μm (PV0.1–0.5), and 0.7–2.5 μm (PV0.7–2.5).

In addition, 24-hr indoor and outdoor fine mass samples were collected on quartz fiber filters for elemental carbon/organic carbon (EC/OC) analysis. We used a parallel plate denuder containing carbon-impregnated papers for the spring–summer 1998 study homes to remove vapor-phase organic carbon before particle collection; use of this denuder was discontinued after extensive field testing indicated that the denuder efficiency was significantly > 99% (34). EC/OC samples were analyzed by thermal/optical reflectance (TOR) (35) at the Desert Research Institute (Reno, Nevada).

Other compositional data collected include total particle-bound polycyclic aromatic hydrocarbons (PAHs). PAHs were measured continuously using an Ecochem PAH monitor (Model 1002i; Ecochem Corporation, West Hills, CA), which sampled in 5-min intervals from the indoor/outdoor manifold. This instrument has been demonstrated to provide semiquantitative measurements through the photoelectric ionization of surface-bound PAHs (6,36–38). The instrument signal was output as a current (in units of picoamperes), but was approximately converted to a concentration (nanograms per cubic meter) using a universal conversion factor of 1 μg/m³/pA proposed by previous studies (36,38).

Other data collected during the comprehensive sampling activities include continuous air exchange rates and detailed time–activity information. Air exchange rates were measured in each home every 5 min using a sulfur hexafluoride tracer gas technique (2,7,39). This technique employs an SF₆ source that releases the gas into the home at a constant source emission rate, and the home volume source event in Home NEW2 (26.6 and 27.5 mg/m³, respectively. As described by Long et al. (2), large indoor/outdoor differences such as these are due to the contributions of indoor source events such as cooking and cleaning activities. Both indoor and outdoor mean PM₂⋅₅ concentrations were highest during the July sampling event in Home NEW2 (26.6 and 27.5 mg/m³, respectively). Relatively high mean indoor concentrations of 17.6 and 17.3 were also observed during the spring and winter sampling events in Home WELL1 despite lower ambient concentrations of 11.3 and 8.4 mg/m³, respectively. As described by Long et al. (2), large indoor/outdoor differences such as these are due to the contributions of indoor source events such as cooking and cleaning activities. Both indoor and outdoor mean PM₀.₃₅–₀.₅ and PM₀.₇–₂.₅ concentrations were highly correlated (Spearman R = 0.72 and 0.91; p < 0.004 and < 0.0001, respectively; Table 2). However, the correlation for the PM₀.₀₂–₀.₁ data was lower and insignificant (R = 0.51; p = 0.06), and in contrast to PM₀.₃₅–₀.₅ and PM₀.₇–₂.₅ data, indoor PM₀.₀₂–₀.₁ concentrations on average were greater than outdoor data. As these are due to the contributions of indoor source events such as cooking and cleaning activities. Both indoor and outdoor mean PM₀.₃₅–₀.₅ and PM₀.₇–₂.₅ concentrations were highly correlated (Spearman R = 0.72 and 0.91; p < 0.004 and < 0.0001, respectively; Table 2). However, the correlation for the PM₀.₀₂–₀.₁ data was lower and insignificant (R = 0.51; p = 0.06), and in contrast to PM₀.₃₅–₀.₅ and PM₀.₇–₂.₅ data, indoor PM₀.₀₂–₀.₁ concentrations on average were greater than outdoor

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concentrations for these study homes. These findings again reflect the impacts of indoor source events, which have been shown to be more pronounced for ultrafine particles (2).

As described previously (2), indoor mean organic carbon concentrations were significantly larger than outdoor concentrations (means of 7.8 and 3.0 µg/m³, respectively), suggesting that indoor particle events may be important sources of indoor organic carbon. Due to the impact of indoor organic carbon sources, there was very little correlation between indoor and outdoor concentrations ($R = 0.02$; Table 2). In contrast, mean indoor and outdoor elemental carbon concentrations were very similar (0.88 and 0.99 µg/m³, respectively) and highly correlated ($R = 0.77$; $p = 0.0014$). Indoor and outdoor mean PAH concentrations were also extremely well correlated ($R = 0.99$; $p < 0.0001$), suggesting that there were few important indoor PAH sources in the study homes.

Although not statistically significant, indoor endotoxin levels were on average higher than outdoor levels (Figure 1, Tables 2 and 3). The indoor and outdoor mean endotoxin concentrations were $3.3 \pm 1.3$ and $2.0 \pm 4$ EU/mg, respectively. The maximum endotoxin concentration was 18.1 EU/mg (Table 3). The indoor/outdoor correlation was low ($R = 0.18$; $p = 0.57$) and the median indoor/outdoor ratio for matching data from each home was 1.5, both suggesting the potential importance of indoor endotoxin sources. Despite the small number of samples ($n = 13$), outdoor endotoxin concentrations were significantly higher ($p = 0.007$) in the two homes sampled in the spring, which might be due to elevated plant emissions during the growing season (40).

**Overview of indoor versus outdoor toxicity responses.** Figure 2 shows a comparison of the TNF release of unprimed AMs for the indoor and outdoor PM$_{2.5}$ samples as well as the negative and positive controls. Table 3 summarizes TNF releases of unprimed AMs by house and season where data have been averaged for the two rats.

We detected TNF only in one of four negative controls (14 pg/mL), whereas TNF releases for indoor and outdoor samples were on average $> 200$ and $> 100$ pg/mL, respectively. On average, the response of the indoor samples was just slightly higher than that for the UAP positive controls ($279 \pm 78$ pg/mL vs. $270 \pm 54$ pg/mL). When indoor and outdoor data were normalized for endotoxin levels, the mean indoor response was still nearly twice as high as the mean outdoor response ($952 \pm 157$ and $494 \pm 96$ pg/EU, respectively). The disparity between indoor/outdoor data remained for the endotoxin-normalized data despite the fact that normalization for endotoxin levels changed the relative ranks of many of the data (Table 3). For example, the average TNF release for the spring NEW1 indoor sample (1,524 pg/mL) was nearly 3 times greater than the next largest response for the unadjusted data. After normalization for endotoxin, it was reduced to a value (840 pg/EU) less than the indoor mean.

For both the unadjusted and endotoxin-normalized data, Wilcoxon signed-rank tests of paired indoor and outdoor samples showed that indoor TNF releases were significantly higher than the corresponding outdoor data ($p = 0.045$ and 0.01, respectively). Indoor–outdoor correlations confirmed the poor relationship between indoor and outdoor data (Figure 3). In contrast to such particulate properties as elemental carbon, PAHs, PV$_{0.1-0.5}$, and PV$_{0.7-2.5}$ (Table 2), there was little correlation between indoor and outdoor toxicity responses. Nonsignificant correlations of $-0.20$ ($p = 0.51$) were obtained for both unadjusted and endotoxin-normalized data. As mentioned above, low and insignificant correlations were also observed for organic carbon and endotoxin, each of which has been demonstrated to have important indoor sources (2,40–44).

As shown by Figures 2 and 3, indoor TNF releases were also more highly variable than outdoor TNF releases. A two-way ANOVA model, which included rat as a blocking factor to control for variability in TNF releases among individual rats, was used to statistically test the sample-to-sample variability of indoor and outdoor samples. Although several homes were sampled twice in different seasons, each sample was treated as an independent sample because ambient and home conditions differed between the two sampling seasons in the same home. Despite the small sample size, the variability of both the indoor-unadjusted and endotoxin-normalized data was found to be statistically significant ($p < 0.0001$ and 0.04, respectively). However, for both sets of outdoor data, the between-sample variability was insignificant ($p = 0.27$ and 0.49 for the unadjusted and endotoxin-normalized data, respectively). These findings for the outdoor data suggest that the significant indoor between-sample variability is not due to differences in ambient particle toxicity. Instead, given that each bioassay was conducted for the same particle concentration (100 µg/mL), differences in indoor particle properties such as composition are likely responsible for the observed indoor-between-sample variability.

Similar to previous studies (23,29), TNF production was significantly elevated for lipopolysaccharide-primed cells, both for negative controls as well as for particle suspensions (data not shown). For the negative controls, priming elicited a mean response of $1,302 \pm 349$ pg/mL, which is three orders of magnitude higher than that for the unprimed controls. The priming effect was even more

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**Table 2. Summary statistics for indoor/outdoor particulate data.**

| Parameter | No. | Location | Particulate concentration data | Indoor/outdoor correlations | Spearman $R$ | $p$-Value |
|-----------|-----|----------|-------------------------------|-----------------------------|-------------|----------|
| PM$_{2.5}$ (µg/m³) | 14 | In | Mean ± SE | Min | Median | Max | 0.51 | 0.06 |
| | | Out | | | | | |
| | 14 | In | 11.8 ± 1.9 | 5.7 | 10.7 | 26.6 | | |
| | 14 | Out | 11.1 ± 1.5 | 4.1 | 10.3 | 27.5 | | |
| PV$_{0.2-0.5}$ (µm³/cm³) | 14 | In | 0.68 ± 0.10 | 0.18 | 0.54 | 1.4 | 0.51 | 0.06 |
| | | Out | 0.48 ± 0.05 | 0.23 | 0.49 | 0.85 | | |
| PV$_{0.1-0.5}$ (µm³/cm³) | 14 | In | 6.2 ± 0.90 | 1.7 | 6.2 | 14.7 | 0.72 | 0.004 |
| | | Out | 6.3 ± 0.94 | 2.1 | 5.8 | 15.0 | | |
| PV$_{0.7-2.5}$ (µm³/cm³) | 14 | In | 2.3 ± 0.45 | 0.85 | 1.9 | 6.5 | 0.91 < 0.0001 | |
| | | Out | 2.7 ± 0.49 | 1.1 | 2.0 | 8.0 | | |
| EC (µg/m³) | 14 | In | 0.88 ± 0.09 | 0.39 | 0.90 | 1.5 | 0.77 < 0.0014 | |
| | | Out | 0.99 ± 0.10 | 0.47 | 0.96 | 1.6 | | |
| OC (µg/m³) | 14 | In | 7.8 ± 0.57 | 4.7 | 7.8 | 13.6 | 0.02 | 0.95 |
| | | Out | 3.0 ± 0.19 | 1.4 | 3.2 | 4.2 | | |
| PAH (ng/m³) | 14 | In | 31 ± 5.6 | 4.2 | 25 | 92 | 0.99 < 0.0001 | |
| | | Out | 27 ± 7.7 | 5.6 | 27 | 107 | | |
| Endotoxin (EU/mg) | 13 | In | 3.3 ± 1.3 | 0.51 | 2.2 | 18.1 | 0.18 | 0.57 |
| | | Out | 2.0 ± 0.41 | 0.36 | 1.5 | 5.0 | | |

Abbreviations: EC, elemental carbon; Max, maximum; Min, minimum; OC, organic carbon.

*All concentrations and correlations are for data that have been averaged over the duration of the sampling period within a home to match the sampling duration of the corresponding toxicity samples. PV$_{0.2-0.5}$ data are not available for the spring sampling event in Home NEW1 due to instrument failure. Endotoxin data are not reported for Home MANI due to extremely low sample recoveries (see text).
amplified in the presence of particles, as the average indoor and outdoor primed responses were over 3,500 and 2,500 pg/mL, respectively. These results demonstrate the enhanced sensitivity of primed cells to both indoor and outdoor fine particles, which has been shown previously for CAP samples (23,29). Given the similarity of the indoor/outdoor relationship for the unprimed and primed data, our focus for the remainder of this paper is on the unprimed bioassay data.

**Evidence of endotoxin-induced TNF production.** Figure 4A and B shows the relationship between endotoxin concentrations and TNF responses for the indoor and outdoor data. These plots show that endotoxin levels were strongly associated with TNF responses for both indoor and outdoor fine particle data. The indoor regression was performed with and without the extreme data point for the spring Home NEW1 sample. This sample yielded the highest endotoxin concentration (18.1 EU/mg) as well as the highest mean TNF responses for both indoor and outdoor data. As mentioned above, the indoor slope was approximately 80; however, the outdoor slope was only 49. The difference between the two slopes suggests that the magnitude of the endotoxin-mediated toxicity response may depend on other particle properties. The synergistic interactions between endotoxin and other proinflammatory components of environmental particles have been previously hypothesized (23,28). In a series of bioassay experiments, Imrich et al. (23) demonstrated that there was no difference in TNF release between primed cells treated with saline (control) or with inert TiO2 particles. However, TNF releases were highly amplified when primed cells were treated with CAPs (23). Furthermore, Ning et al. (28) showed that particle-associated endotoxin in CAP samples elicits much greater bioactivity than the same amount of soluble endotoxin given to AMs alone. For this study, the larger indoor slope suggests that there is greater synergy between endotoxin and components of indoor particles.

**Influence of air exchange rate on indoor particle toxicity.** Figure 5A and B shows that higher indoor TNF releases and indoor/outdoor differences in TNF releases were typically observed in homes with lower air exchange rates, particularly for the endotoxin-normalized data. Air exchange rates were classified as either high or low on the basis of whether they were above or below the median home air exchange rate of 0.84/hr. Despite the small sample size, both indoor endotoxin-normalized TNF releases, as well as the difference between indoor/outdoor endotoxin-normalized TNF releases, were significantly higher for the low air exchange rate class (p = 0.005 for both). Similar Wilcoxon ranked-sum tests of the unadjusted data yielded insignificant p-values of 0.23 and 0.14, respectively. These findings, together with evidence that the impacts of indoor source events are even more pronounced at low air exchange rates when indoor residence times are longer and indoor-generated particles can accumulate (2,7,49), suggest that the higher indoor toxicity responses may be due to the effects of indoor-generated particles.

**Estimation of indoor toxicity components.** We constructed a simple physical–statistical model to quantify the relative contributions of indoor-generated and ambient particles to the indoor toxicity response. This model assumes that the endotoxin-normalized indoor toxicity response (Tox-in in picograms per endotoxin unit) is a function of the fraction of particles of indoor origin (F-in) and those of ambient origin (F-a):

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Tox_{-in} = \alpha_{in}F_{-in} + \alpha_{a}F_{-a},
\]

where \( \alpha_{in} \) and \( \alpha_{a} \) represent the portions of the indoor toxicity response that can be attributed to particles of indoor origin and those of ambient origin, respectively. This model is based on the fact that each bioassay was conducted for a uniform exposure concentration of 100 µg/mL, thus effectively removing any relationship with particle concentration. We also assumed that \( F_{-in} \) and \( F_{-a} \) represent the fraction of indoor-generated and ambient particles, respectively, in the 100 µg/mL particle suspensions. We used endotoxin-normalized data in the model because we hypothesized that there are synergistic interactions between particles and endotoxin which depend on particle properties.

### Table 3. Summary of PM2.5 endotoxin and toxicity data by house and season.

| Home ID | Season | Indoor (EU/mg) | Outdoor (EU/mg) | Unadjusted (pg/mL) Indoor | Unadjusted (pg/mL) Outdoor | Normalized (pg/EU) Indoor | Normalized (pg/EU) Outdoor |
|---------|--------|----------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------|
| MAN1    | Winter | —              | —              | —                         | —                         | —                         | —                         |
| NEW1    | Spring | 10.135         | 4.989          | 1524                      | 64                        | 840                       | 127                       |
|          | Fall   | 2.018          | 1.463          | 221                       | 41                        | 1,093                     | 277                       |
| WEL1    | Spring | 1.049          | 4.477          | 55                        | 389                       | 520                       | 869                       |
|          | Winter | 1.304          | 0.937          | 218                       | 18                        | 1,668                     | 192                       |
| SWP1    | Summer | 2.465          | 2.861          | 71                        | 87                        | 288                       | 302                       |
| BOX1    | Summer | 4.737          | 0.648          | 558                       | 25                        | 1,177                     | 378                       |
| NEW2    | Summer | 0.819          | 0.355          | 135                       | 209                       | 466                       | 958                       |
|          | Fall   | 2.996          | 2.182          | 37                        | 257                       | 1,123                     | 2,150                     |
| FOX1    | Summer | 1.368          | 0.678          | 101                       | 18                        | 735                       | 258                       |
|          | Winter | 2.179          | 0.698          | 324                       | 37                        | 1,485                     | 530                       |
| WEL2    | Summer | 0.511          | 2.748          | 134                       | 178                       | 2,613                     | 646                       |
| SWP2    | Winter | 2.16           | 1.355          | 227                       | 60                        | 1,051                     | 443                       |

Mean ± SE

| Home ID | Season | Indoor (EU/mg) | Outdoor (EU/mg) | Unadjusted (pg/mL) Indoor | Unadjusted (pg/mL) Outdoor | Normalized (pg/EU) Indoor | Normalized (pg/EU) Outdoor |
|---------|--------|----------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------|
| MAN1    | Winter | —              | —              | —                         | —                         | —                         | —                         |
| NEW1    | Spring | 10.135         | 4.989          | 1524                      | 64                        | 840                       | 127                       |
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|          | Fall   | 2.996          | 2.182          | 37                        | 257                       | 1,123                     | 2,150                     |
| FOX1    | Summer | 1.368          | 0.678          | 101                       | 18                        | 735                       | 258                       |
|          | Winter | 2.179          | 0.698          | 324                       | 37                        | 1,485                     | 530                       |
| WEL2    | Summer | 0.511          | 2.748          | 134                       | 178                       | 2,613                     | 646                       |
| SWP2    | Winter | 2.16           | 1.355          | 227                       | 60                        | 1,051                     | 443                       |

Mean ± SE

**Figure 2.** TNF production of unprimed AMs in negative controls (n = 4), positive controls (UAP; n = 4), and indoor (n = 26) and outdoor (n = 26) PM2.5 samples. (A) Unadjusted TNF data. (B) Endotoxin-normalized TNF data.
Because \( F_i = 1 - F_{in} \), Equation 1 is simply equal to the following:

\[
\text{Tox}_\text{in} = \alpha_0 F_{in} + \alpha_4 (1 - F_{in}) \tag{2}
\]

After rearranging terms, the following equation is obtained:

\[
\text{Tox}_\text{in} = (\alpha_0 - \alpha_4) F_{in} + \alpha_4 \tag{3}
\]

If \( \text{Tox}_\text{in} \) is regressed on \( F_{in} \), the slope (e.g., \( \alpha_0 - \alpha_4 \)) approximates the difference between the mean endotoxin-normalized TNF response attributable to indoor particles and that for indoor particles of ambient origin, whereas the intercept (e.g., \( \alpha_4 \)) represents the mean response due to indoor particles of ambient origin.

We calculated model values for \( F_{in} \) for these study homes using previously reported estimates of ambient particle infiltration factors (\( F_{in,0} \)) (46). Infiltration factors, which ranged from 0.40 to 1.09, were estimated from simultaneous indoor/outdoor PM\(_{2.5}\) data from nighttime, nonsource periods for all but the spring sampling events in Homes WEL1 and NEW1 (46). Nightly \( F_{in,0} \) estimates were averaged over the entire sampling duration within a home to match the averaging period of the toxicity data. For the spring sampling events in Homes WEL1 and NEW1, where matching indoor/outdoor continuous PM\(_{2.5}\) measurements were not available, SMPS and APS particle volume data were summed to approximate PV\(_{2.5}\).

The indoor concentration of ambient fine particles (\( C_i \)) was first quantified by multiplying the infiltration factor by the outdoor PM\(_{2.5}\) concentration (\( C_{out} \)):

\[
C_i = F_{in,0} \times C_{out} \tag{4}
\]

It was then possible to estimate the indoor fraction of ambient particles

\[
F_i = \frac{C_i}{C_{in}} \tag{5}
\]

and the corresponding indoor fraction of indoor-generated particles

\[
F_{in} = 1 - F_i \tag{6}
\]

Estimates of \( F_{in} \) ranged from a low of approximately 0 for the summer sampling events in Homes SWP1, BOX1, and NEW1 to a high of 0.74 for the winter sampling event in Home WEL1 (0.28 ± 0.06, mean ± SE).

Model results presented in Figure 6A and B suggest the enhanced bioactivity of indoor-generated particles. Despite the small data set, we found a strong and near-significant relationship (\( R^2 = 0.20; p = 0.06 \)) between the endotoxin-normalized TNF release and \( F_{in} \) (Figure 6A). Furthermore, the intercept of 491 ± 275 pg/EU, which represents the mean endotoxin-normalized TNF response attributable to indoor particles of ambient origin, was very close to the outdoor mean TNF release of 494 ± 96 pg/EU. In contrast, the estimate for the mean endotoxin-normalized TNF response attributable to indoor-generated particles was approximately 2,100 ± 600 pg/EU (Figure 6B).

**Discussion**

Rat AMs treated with either indoor or outdoor PM\(_{2.5}\) released significant amounts of TNF compared to control AMs. Furthermore, these TNF releases for both indoor and outdoor PM\(_{2.5}\) samples are of similar magnitude to that observed for CAPs by other investigators. Specifically, Imrich et al. (23) observed mean TNF releases of 10–130 pg/mL for rat AMs exposed to 100 µg/mL CAPs from several daily samples, and 20–260 pg/mL for human alveolar macrophages exposed to 50 µg/mL CAP suspensions. In addition, TNF production was further amplified for primed AMs exposed to these indoor and outdoor PM\(_{2.5}\) samples. This priming effect is comparable to that observed by a previous study of rat and human AMs exposed to CAPs or UAP (23). These results suggest that indoor PM\(_{2.5}\) may also have a synergistic effect on the inflammatory response in people with preexisting proinflammatory conditions.

A comparison of paired indoor/outdoor data demonstrated that significantly greater TNF releases were elicited by indoor PM\(_{2.5}\) samples than by the corresponding outdoor samples. The significance of this indoor/outdoor difference slightly increased when data were normalized for endotoxin concentrations. This finding alone suggests that indoor particles are at least as toxic as outdoor particles. As described earlier, indoor particles include particles of both ambient and indoor origin. Together with this indoor/outdoor difference, other study findings suggest that particles of indoor origin may be more bioactive than particles of ambient origin.

The role of indoor-generated particles in indoor particle bioactivity is supported by several study findings. Specifically, the low indoor/outdoor correlation between paired toxicity data is suggestive of the impact of indoor-generated particles. Also, regressions of TNF releases on endotoxin concentrations yielded steeper slopes for indoor than outdoor data, suggesting that there may be greater synergism between endotoxin and components of indoor particles. In addition, indoor but not outdoor TNF releases were

![Figure 3](image-url) **Figure 3.** Indoor versus outdoor TNF releases for (A) unadjusted data (Spearman \( R = –0.20; p = 0.51; n = 13 \)) and (B) endotoxin-normalized data (Spearman \( R = –0.20; p = 0.51; n = 13 \)). Each data point represents data from one sampling event in which TNF releases have been averaged over the two rats (\( n = 13 \)).

![Figure 4](image-url) **Figure 4.** Linear regressions of TNF release versus endotoxin concentration for (A) indoor data (regression output: slope = 84 (\( p < 0.01 \)); \( R^2 = 0.92 \), without extreme data point: slope = 82 (\( p = 0.03 \)); \( R^2 = 0.40 \)) and (B) outdoor data (regression output: slope = 49 (\( p = 0.02 \)); \( R^2 = 0.38 \)).
shown to exhibit significant between-sample variability. Due to the use of a uniform exposure concentration of 100 µg/mL, the variability in indoor TNF releases may be attributed to fluctuations in particle properties (e.g., composition, size) among the indoor samples. The contributions of indoor source events have been previously shown to be a dominant source of variability for indoor particle concentrations and size distributions (2,7). Furthermore, it has been previously reported that source strengths of indoor particle events were highly variable in these study homes (2). If differences in indoor source types and event frequency among the study homes are also considered, it is likely that variability in indoor particle emission rates, as well as particle characteristics among the study homes, may explain the variability in indoor TNF releases.

It has also been reported that the impacts of indoor particle events are amplified under conditions of low air exchange rate (2,7,45). Results from the present study have shown that the level of indoor bioactivity and the difference between indoor and outdoor bioactivities depend on air exchange rate. The five largest indoor/outdoor differences in the endotoxin-normalized TNF release occurred for homes with lower exchange rates (i.e., below the study median), suggesting that the indoor toxicity response is amplified when air exchange rates are low and indoor residence times are high. During low air exchange rate conditions, indoor particle events can dramatically increase the fraction of indoor particles of indoor origin as concentrations of indoor-generated particles build up. In addition, low air exchange rates are also associated with decreased ambient particle infiltration (46–49), which results in diminished indoor concentrations of ambient particles. Thus, it would appear that conditions which favor the accumulation of indoor-generated particles rather than ambient particles may thus raise the toxicity of indoor particles.

Despite the small sample size, the results of our simple physical–statistical model confirm that indoor particle toxicity may be elevated as the fraction of indoor-generated particles increases. This model demonstrated that differences in the indoor toxicity response between samples could be explained by the fractions of particles of indoor and ambient origin. The mean endotoxin-normalized TNF response attributable to particles of indoor origin was over four times higher than the corresponding estimate for particles of ambient origin (2,100 ± 600 pg/EU versus 491 ± 275 pg/EU).

It is still unclear which components of indoor-generated particles may be responsible for their enhanced bioactivity. In this study we have confirmed the role of endotoxin as a stimulant of cytokine production (17,23,25,28). However, endotoxin levels were not found to differ significantly between indoor and outdoor fine particles. In addition, normalization for endotoxin did not eliminate the variability in the indoor TNF data, suggesting that there are other proinflammatory components of indoor particles. Based on in vitro experiments employing endotoxin inhibitors, other investigators have hypothesized that there are other proinflammatory components of ambient particles (28,29). One possible proinflammatory component for indoor particles may be organic carbon, which was present in significantly higher concentrations in indoor PM2.5 samples. Organic carbon is known to be enriched in fine particles, and previous studies have demonstrated the mutagenic (50–52) and carcinogenic (53) properties of airborne particulate-bound carbon.

Although these results are suggestive, caution must be exercised in interpreting them. The implications of these results with respect to in vivo effects are very uncertain. In vitro exposure conditions are clearly not representative of particle inhalation and deposition in the lungs. In addition, although TNF is known to initiate the inflammatory activation of AMs, it is unclear whether the differences in TNF release for indoor versus outdoor PM2.5 would result in different in vivo toxic effects. Furthermore, these study findings are also based on a small sample size. PM2.5 samples were collected from only nine homes and represent very small periods of time in these homes (e.g., 1–2 weeks).

The intent of this study was to explore the relative toxicities of indoor and outdoor fine particles. These study findings indicate that particles of indoor origin can induce cytokine production, and they point to the need for additional efforts to understand indoor exposures to both particles of indoor origin as well as those of ambient origin. Given the large amounts of time that people spend indoors, these study findings suggest that indoor particles should be the focus of further toxicologic research.

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