Ambient Endotoxin Concentrations in PM_{10} from Southern California

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Concentrations of endotoxin in urban air pollution have not previously been extensively characterized. We measured 24-hr levels of PM_{10} (particulate matter < 10 μm in aerodynamic diameter) and the associated endotoxin component once every 6 weeks for 1 year in 13 communities in Southern California. All the samples collected had detectable PM_{10} and endotoxin levels. The geometric mean PM_{10} was 34.6 μg/m^3 [geometric SD (GSD), 2.1; range, 3.0–135]. By volume, the endotoxin geometric mean was 0.44 endotoxin units (EU)/m^3 (GSD, 3.1; range, 0.03–5.44). Per unit material collected, the geometric mean of endotoxin collected was 13.6 EU/mg (GSD, 3.2; range, 0.7–96.8). No correlation was found between endotoxin concentrations and other ambient pollutants concurrently measured [ozone, nitrogen dioxide, total acids, or $PM_{2.5}$ (particulate matter < 2.5 μm in aerodynamic diameter]. PM_{10} and endotoxin concentrations were significantly correlated, most strongly in summer. Samples collected in more rural and agricultural areas had lower PM_{10} and mid-range endotoxin levels. The high desert and mountain communities had lower PM_{10} levels but endotoxin levels comparable with or higher than the rural agricultural sites. By volume, endotoxin levels were highest at sites downwind of Los Angeles, California, which were also the locations of highest PM_{10}. Endotoxin concentrations measured in this study were all < 5.5 EU/m^3, which is lower than recognized thresholds for acute adverse health effects for occupational exposures but in the same range as indoor household concentrations. This study provides the first extensive characterization of endotoxin concentration across a large metropolitan area in relation to PM_{10} and other pollutant monitoring, and supports the need for studies of the role of endotoxin in childhood asthma in urban settings. *Key words:* **air pollution, bioaerosol, endotoxin, lipopolysaccharide, particulate matter.** *Environ Health Perspect* **112:583–588 (2004). doi:10.1289/ehp.6552 available via** *http://dx.doi.org/* **[Online 14 January 2004]**

It has been increasingly recognized through epidemiologic investigations that particulate matter (PM) in agricultural air contributes to the progression and exacerbation of respiratory diseases such as asthma, and in urban air leads to an increase in morbidity and mortality from respiratory and cardiac conditions (Dockery 2001; Fairley 1999; Ostro et al. 1999, 2000; Pope 1999, 2000; Pope et al. 1995; Samet et al. 2000a, 2000b). Furthermore, ambient exposure to PM has been associated with adverse effects on childhood lung function growth, which theoretically could increase lifetime risk for chronic respiratory disorders (Gauderman et al. 2000, 2002; Jedrychowski et al. 1999). Environmental air pollution may be especially injurious to infants, children, and adolescents because of *a*) their increased ventilation rates; *b*) their physical, temporal and spatial activity patterns; and *c*) the fact that their lungs are rapidly growing and developing (Peters et al. 1999b; Plopper and Fanucchi 2000).

The exact constituents of air pollution that cause disease and the precise mechanisms involved are complex. Numerous studies have been conducted to determine which components of PM may contribute to airway inflammation and irritation (Bonner et al. 1998; Donaldson and MacNee 2001; Li et al. 1997; Monn and Becker 1999; Ning et al. 2000; Soukup and Becker 2001). Various aerodynamic PM size fractions have also been studied, including PM < 10 μm in aerodynamic diameter (PM_{10}), PM < 2.5 μm ($PM_{2.5}$), and submicrometer-sized fractions < 1.0 μm in aerodynamic diameter; PM_1). Recent research has focused on the associated health effects of the fine and submicrometer fractions, which are made up primarily of anthropogenic emissions (Lippmann and Schlesinger 2000; Pope 2000). However, the coarse PM fraction (PM_{10}, in this context) is recognized as having significant adverse effects on the bronchiolar region of conducting airways—the primary site of asthma and associated airway inflammation (Monn and Becker 1999; Soukup and Becker 2001).

One component of the PM_{10} fraction of particular interest is endotoxin. Endotoxin is a lipopolysaccharide (LPS) component of the cell wall of gram-negative bacteria that, when inhaled, stimulates alveolar macrophages and respiratory epithelial tissue to release cytokines—chemoattractants that initiate an inflammatory cascade (Thorne 2000). Human exposure–response studies have demonstrated a decline in airflow, development of neutrophilic alveolitis, and increased cytokine release by activated macrophages and airway epithelial cells upon inhalation exposure to endotoxin (Clapp et al. 1994; Jagielo et al. 1996; Kline et al. 1999). Previous studies have shown that endotoxin is the most significant component associated with the development and progression of airway disease in workers exposed to organic dust (Schwartz et al. 1995). Endotoxin is well recognized as an occupational hazard in livestock confinement barns and grain handling facilities and during harvesting of row and specialized crops, cotton processing, vegetable washing, sawmills, metal machining, fiberglass production, composting, and waste handling (Douwes et al. 2003a, 2003b).

Similarly, endotoxin concentrations in the indoor home environment have been linked to adverse respiratory health effects. Although some studies have suggested a protective role of endotoxin exposure in infancy, exposure to endotoxin in childhood and later in life appears to have a detrimental effect in both healthy volunteers and in individuals with asthma and other respiratory conditions (Douwes and Heederik 1997; Douwes et al. 2002; Michel et al. 1996). In childhood, endotoxin exposure is associated with increased wheezing and exacerbation of asthma (Douwes et al. 2000; Park et al. 2001a; Rizzo et al. 1997). Several studies have shown that individuals with asthma develop airflow obstruction at lower concentrations of inhaled endotoxin than do normal individuals (Kline et al. 1999; Michel et al. 1989). One such study found that endotoxin exposure is more significantly associated with the clinical severity of asthma than is exposure to allergen concentrations alone (Michel et al. 1996).

Although several sources of indoor endotoxin have been described (Heinrich et al. 2001; Park et al. 2001b; Wouters et al. 2000), the contribution of endotoxin from the outdoor environment has not been well

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characterized (Menetrez et al. 2001). During warmer months of the year, and in more temperate climates, it is possible that outdoor endotoxin levels have an influence on indoor levels, especially when the windows are open or if the building is otherwise not tightly sealed (Park et al. 2000).

Recent recognition of appreciable levels of indoor endotoxin in residences nationwide (Thorne et al. 2003a) motivated the present study, which characterizes outdoor ambient levels of endotoxin. As with ambient air pollution, children may be more susceptible to endotoxin in the outdoor environment because of reasons mentioned above. Furthermore, there may be additional or synergistic effects of coincident exposure to both endotoxin and other components of PM_{10}. One study suggested that exposure to endotoxin may prime macrophages, resulting in a more vigorous inflammatory response upon exposure to other anthropogenic components of PM, particularly in patients with underlying inflammatory lung diseases such as asthma (Imrich et al. 1999).

The goals of the study were to determine ambient endotoxin levels in a variety of communities in Southern California with differing climactic profiles, degrees of urbanization, and air pollution levels; to characterize seasonal variability of ambient endotoxin in these same communities; and to see how endotoxin levels correlate with the ambient coarse particle fraction (PM_{10}).

Materials and Methods

Sampling locations. The communities in which ambient sampling was performed (Figure 1) were the same as or adjacent to communities participating in the Children's Health Study (CHS), a multiyear prospective cohort study of the chronic effects of air pollution on the respiratory health of more than 6,000 California schoolchildren across six Southern California counties (McConnell et al. 1999; Peters et al. 1999b). The CHS investigation involves both annual health testing of participating schoolchildren and continuous daily monitoring of ambient gaseous and particulate pollutants, to develop long-term averages of pollution levels in the respective communities (Gauderman et al. 2000; Peters et al. 1999a). Study communities included coastal, mountainous, high desert, urban, and rural locations up to 300 km north, east, or south of Los Angeles, California. For the present study, local regulatory monitoring agency air-monitoring stations were used as the community sampling location.

Specific community selections were based on the presence or proximity of a regulatory agency air-monitoring station in or near a CHS community, access to and availability of a Federal Reference Method PM_{10} filter sampler at the station of interest, and the cooperation and willingness of the local agency field personnel to operate and maintain the field sampling program as directed by study investigators.

Air sampling. High-purity quartz microfiber filters (20.3 cm × 25.4 cm; Whatman International, Ltd., Maidstone, England) were equilibrated overnight on racks at ambient temperature and humidity in an environmentally controlled gravimetrics laboratory and then weighed on a calibrated Mettler balance (Mettler Instrument Corp., Hightstown, NJ). Before weighing the filters, a balance check was performed using NIST standard weights (National Institute of Standards and Technology, Gaithersburg, MD). Filters were inspected for tears, folds, and other imperfections, and the serial number was recorded. After weighing every filter, 10% of the filters were randomly chosen to be reweighed as a quality control check. If any of the second weights differed by more than ± 5 mg from the original weight, all filters in that set were reweighed.

After the weight was recorded, the filter was immediately placed in a new, clean Tyvek envelope prelabeled with the corresponding filter serial number and sampling site destination. Envelopes were then sealed and placed inside a larger mailing envelope along with a custody sheet labeled with the corresponding serial number and sampling site name. The filters were express-mailed from Iowa to their respective sampling sites. For every sampling date, one additional filter was sent to each of two randomly ordered sites to be used as blanks for that sampling round. Over the course of the study, every site received two filters to be used as field blanks.

At the sampling site, the filter was loaded into the collection cassette, and the sampler timer was set to begin collection at midnight of the assigned date. High-volume PM_{10} samples were collected for 24 hr at a calibrated flow rate of approximately 1,132 L/min (40 ft^3/min). Blank filters were handled in the same manner, except they remained inside the station for the collection duration. After collection, filters were removed from the cassette, carefully folded in half to enclose the exposed surface, and placed into the labeled Tyvek return envelope. Collection time, standardized flow rate, and weather conditions were recorded on the custody sheet and were returned with the filter by express mail to the laboratory in Iowa.

Upon receipt, the express mail envelopes containing the filters were placed in a chamber

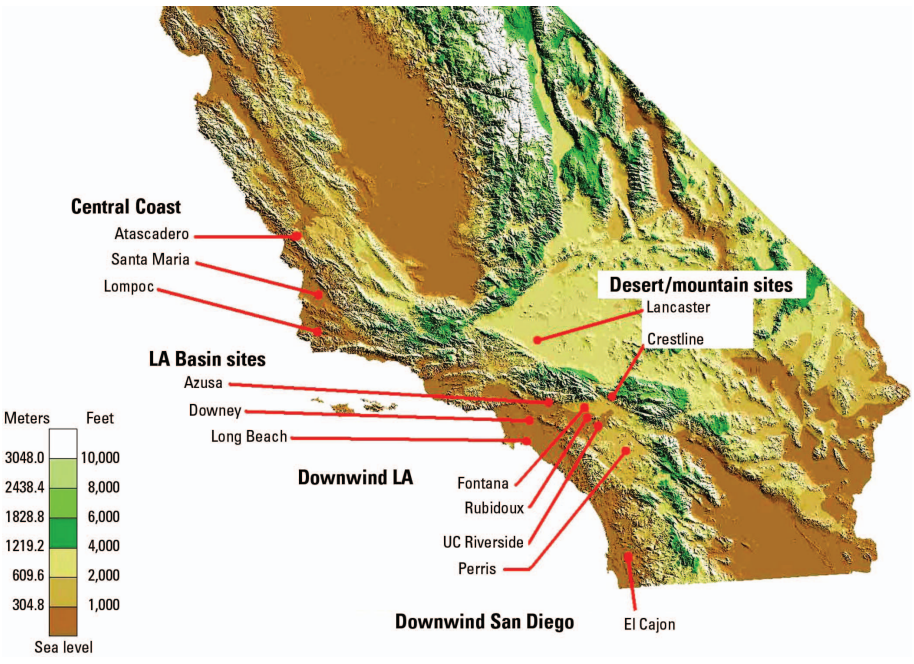

Figure 1. Map of Southern California showing locations of the air-monitoring sites and their geographic groupings. UC, University of California.

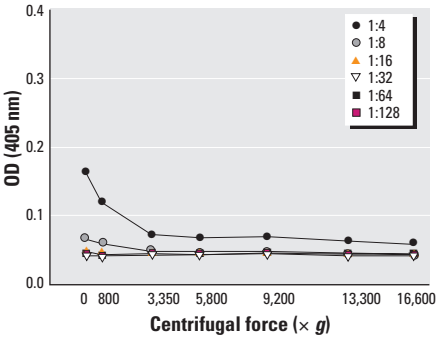

Figure 2. Effect of centrifugation rate on removal of suspended fine particulates (determined by OD) from the filter extraction solutions at various dilutions.

with desiccant and held in a 4°C cold room until all filters had been returned. The day before analysis, the filters were removed from the envelopes, inspected, and equilibrated overnight as described previously. All filters were postweighed, and 10% of the filters were reweighed as a quality control check.

Endotoxin analysis. After reweighing, folded filters were placed on a clean sheet of aluminum foil and cut into 2-cm strips with a sterile scalpel. The strips were then placed into sterile, pyrogen-free 250-mL screw-capped centrifuge bottles (Corning Inc. Life Sciences, Acton, MA) and eluted with 100 mL sterile, pyrogen-free water with 0.05% Tween-20 on a platform shaker (Barnstead International/Lab Line 1314, Dubuque, IA) at maximum rate (220 rpm) for 1 hr. During this time, the bottles were checked every 15 min to ensure that the filter strips remained submerged in the elution fluid. The bottles were then vortexed and filter fragments were allowed to settle. Next, 1.5 mL of the eluant was transferred to screw-capped cryovials (Sarstedt AG & Co., Nümbrecht, Germany) and centrifuged to clear the elution fluid of black particulates that would interfere with the assay. The resulting cleared supernatant was diluted 2-fold from 1:4 to 1:128 and assayed for endotoxin at each dilution using the kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay (BioWhittaker Inc., Walkersville, MD) as previously described (Thorne 2000). Blank filters were assayed undiluted and at a 1:4 dilution. Reagent blanks and a 13-point standard curve using control standard endotoxin were assayed on the same microtiter plate in the same manner as the samples. The absorbance was measured on a microplate reader (SpectraMax 340; Molecular Devices, Sunnyvale, CA) at 405 nm every 30 sec for 90 min. Endotoxin determinations were based on the maximum slope of the absorbance versus time plot for each microplate well compared with the standard curve. Sample concentrations were reported as endotoxin units (EU) per milliliter of eluant, EU per milligram of dust, and EU per cubic meter of air collected.

Statistical analysis. We performed univariate analyses, Pearson correlation analyses, and tests of normality using SAS software (Version 8; SAS Institute Inc., Cary, NC). Multivariate analyses were performed to determine if there were important differences in PM_{10} and endotoxin concentration across geographical regions and over time. This analysis was performed using a repeated-measures analysis (SAS Proc GLM) of the log-transformed data with 99 measured values and 5 imputed values. p-Values < 0.05 were considered significant.

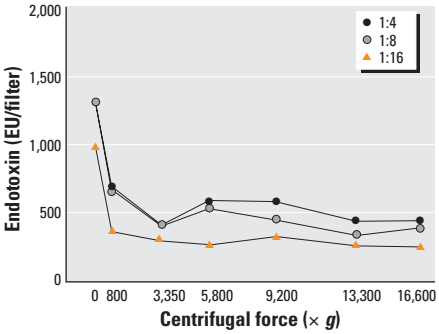

Figure 3. Effect of centrifugal force on endotoxin levels extracted from various dilutions of the filter extraction solutions. Centrifugation forces > 800 × g had little effect on the amount of endotoxin recovered from the sampling filters. Samples run without centrifugation demonstrated interference with the assay because of particle suspensions.

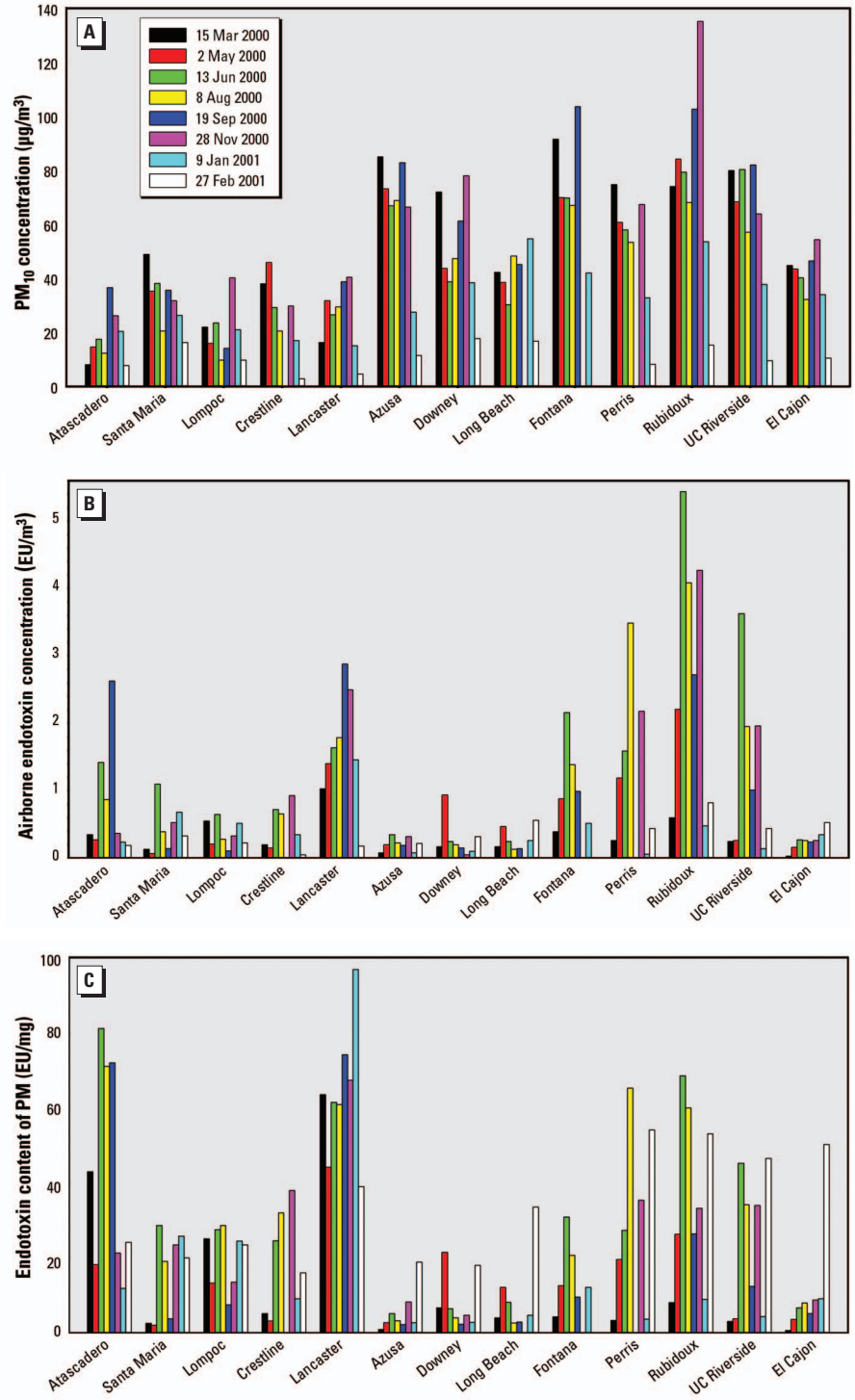

Figure 4. Concentrations of PM and endotoxin for each site and each sampling date. (*A*) PM_{10} concentration. (*B*) Airborne endotoxin concentration. (*C*) Endotoxin content in the collected PM. UC, University of California.

Optimization of the filter extraction procedure. After initial filter elution, extraction solutions were often opaque and contained suspensions of fine black particulates and glass fiber filter debris. This resulted in artificially elevated optical density (OD) readings and interfered with the performance of the kinetic LAL endotoxin assay. A high initial OD value decreases the available data points before the maximum possible OD reading is reached, could potentially alter the shape of the curve, and may interfere with the determination of V_{max} in the absorbance versus time plot. Therefore, we developed a specific protocol to reduce suspended particles while maintaining the endotoxin in solution. Filter eluant (50 mL) was centrifuged at 3,500 × *g* for 10 min to remove pieces of disintegrated filter and facilitate pipetting of the sample into aliquots. One-milliliter aliquots were transferred to seven sampling vials, and the seven vials were microcentrifuged for 20 min at 0, 800, 3,350, 5,800, 9,200, 13,300, or 16,200 × *g*, respectively. The resulting supernatants were individually pipetted onto a microtiter plate, diluted 2-fold from 1:4 up to 1:128, and evaluated for OD at 405 nm (Figure 2). This experiment demonstrated that centrifugation at 5,800 × *g* effectively reduced the baseline OD reading of the filter eluant to a level that would allow the kinetic assay to be performed. Furthermore, the results suggested that dilutions above 1:8 should be preferentially used to further decrease baseline OD. The experiment was repeated to determine the effect of centrifugation on the actual LAL assay. Results demonstrated that a centrifuge force of 5,800 × *g* and sample dilution of ≥ 1:4 were best for endotoxin analysis (Figure 3).

We were also concerned that endotoxin recovery might be diminished through binding of endotoxin to solid particles in the insoluble fraction removed during centrifugation. Addition of a surfactant (Tween-20) and vigorous shaking and vortexing were presumed to be mitigating factors, but a spiking assay was performed to test for recovery of endotoxin activity. Additional filters were collected from two sampling sites with high PM_{10} (Rubidoux and Azusa, California) likely to represent high endotoxin (Rubidoux) and low endotoxin concentrations (Azusa). These samples yielded PM_{10} concentrations of 82 and 44 μg/m^3, respectively, and endotoxin concentrations of 2.38 EU/m^3 and 0.36 EU/m^3, respectively. Exposed filters were cut into quarters, and two opposing quarters from each filter were each spiked with 195 EU endotoxin (LPS from *Escherichia coli* O55:B5, BioWhittaker) in 50 μL pyrogen-free water and allowed to dry in a desiccator. Opposing filter quarters were then extracted together in 50-mL volumes, and the endotoxin on the spiked half was compared with the unspiked half. The recovery of endotoxin from the spiked filters was 100.5% for the filter from Rubidoux and 110.8% for the Azusa sample (mean recovery, 105.6%), well within the acceptable range for endotoxin spiking assays.

Results

Of the 130 filters (104 samples, 26 blanks) sent to our sampling stations, five were not available for analysis because of sampling equipment failures. All PM_{10} samples analyzed yielded quantifiable concentrations of particulates and endotoxin. These data are plotted in Figure 4A–C and summarized in Table 1. Endotoxin and PM_{10} levels of the blank filters were generally at or below the analytical limits of detection, with one exception. For one sampling date, the blank filters gave somewhat higher results for endotoxin. For this case, the mean EU per filter of the blank filters was subtracted from the EU per filter values of the site filters.

By geographic location, rural agricultural sites (at Atascadero, Lompoc, and Santa Maria) had the lowest PM_{10} but were mid-range in terms of endotoxin. The desert and mountain locations (Lancaster and Crestline, respectively) had lower PM_{10} but were toward the upper end of the monitored communities for endotoxin. Los Angeles (LA) Basin locations (Long Beach, Azusa, and Downey) had moderate PM_{10} among the 13 sites and the lowest endotoxin results. Communities in the downwind plume of Los Angeles (Fontana, Rubidoux, Riverside, and Perris) and San Diego (El Cajon) had the highest observed PM_{10} and tended to be in the upper quartile of reporting communities for endotoxin.

No obvious seasonal patterns for endotoxin concentration were detected, but results for many sites suggested higher airborne endotoxin concentration in the months of June through September. Not surprisingly, both PM_{10} and endotoxin levels were lower on days with precipitation. Analysis of variance for repeated measures demonstrated highly significant differences across sampling dates and regions for PM_{10}, airborne endotoxin concentration and for the endotoxin content of the dust (Table 2). However, the interaction of date and region was only significant for endotoxin.

PM_{10} and endotoxin concentrations determined from the same filters were most strongly correlated for samples collected in June (Pearson $r = 0.66$, $p = 0.01$), November ($r = 0.65$, $p = 0.03$), and February ($r = 0.59$, $p = 0.04$). In order to compare the endotoxin data with other ambient air pollutants, we compared annualized endotoxin concentration (EU per cubic meter) with annual concentrations of

Table 1. Summary of geometric mean (range) for the 13 Southern California sampling sites averaged over 1 year.

Region	PM_{10} (μg/m^3)	Airborne endotoxin concentration (EU/m^3)	Endotoxin content of PM (EU/mg)
Central Coast sites	20.3 (7.8–48.9)	0.36 (0.07–2.63)	18.9 (2.1–81.1)
Atascadero	15.8 (7.8–36.6)	0.52 (0.19–2.63)	34.5 (11.9–81.1)
Lompoc	17.7 (9.8–40.3)	0.31 (0.11–0.65)	18.8 (7.5–28.7)
Santa Maria	30.0 (16.3–48.9)	0.30 (0.07–1.10)	10.4 (2.1–28.7)
Desert/mountain sites	21.1 (3.0–45.9)	0.66 (0.05–2.88)	30.0 (3.2–96.8)
Crestline	20.7 (3.0–45.9)	0.30 (0.05–0.93)	13.3 (3.2–38.0)
Lancaster	21.5 (4.6–40.5)	1.30 (0.18–2.88)	61.2 (39.0–96.8)
LA Basin sites	44.8 (11.5–85.1)	0.20 (0.05–0.94)	5.4 (1.0–33.6)
Azusa	51.6 (11.5–85.1)	0.19 (0.08–0.35)	3.8 (1.0–18.9)
Downey	45.7 (17.8–78.1)	0.19 (0.05–0.94)	6.3 (2.4–21.5)
Long Beach	37.2 (16.8–54.7)	0.25 (0.13–0.56)	6.6 (2.7–33.6)
Downwind LA sites	56.4 (8.2–135.1)	1.07 (0.06–5.44)	17.8 (3.1–68.5)
Fontana	71.2 (42.1–103.6)	0.90 (0.39–2.16)	12.6 (4.3–30.9)
Perris	42.6 (8.2–74.8)	0.72 (0.06–3.49)	18.9 (3.4–65.2)
Rubidoux	66.6 (15.4–135.1)	1.85 (0.48–5.44)	27.9 (8.1–68.5)
UC Riverside	51.2 (9.6–82.0)	0.70 (0.14–3.63)	14.1 (3.1–46.5)
Downwind San Diego site	35.1 (10.5–54.4)	0.21 (0.03–0.53)	6.4 (0.7–50.2)
El Cajon	35.1 (10.5–54.4)	0.21 (0.03–0.53)	6.4 (0.7–50.2)
Overall	34.6 (3.0–135.1)	0.44 (0.03–5.44)	13.6 (0.7–96.8)

UC, University of California.

Table 2. Analysis of variance for repeated measures showing that both PM_{10} and endotoxin concentrations differed by region and sampling date.

		PM_{10}		Endotoxin concentration			
				EU/m^3		EU/mg dust	
	df	*F*	*p*-Value	*F*	*p*-Value	*F*	*p*-Value
Region	4	12.06	0.002	6.22	0.014	4.22	0.040
Date	7	24.02	< 0.0001	6.02	< 0.0001	8.80	< 0.0001
Date × region	28	1.13	0.345	3.15	0.0001	2.27	0.0046

df, degrees of freedom.

ambient pollutants measured over the entire 2000 calendar year. Specific pollutants measured included daytime ozone (0600 hr–1000 hr), 24-hr ozone, 24-hr nitrogen dioxide, 24-hr PM_{10}, 24-hr $PM_{2.5}$, and total acids (nitric + formic + acetic + hydrochloric). Of these pollutants, only PM_{10} was significantly correlated with endotoxin concentration ($r = 0.74$, $p = 0.005$). Seasonally, the correlation coefficients between endotoxin and PM_{10} were highest in the summer ($r = 0.72$, $p = 0.008$) and lowest in the winter ($r = 0.33$, $p = 0.29$).

Discussion

Endotoxin concentrations differed significantly across regions and over the course of the year. Geometric mean concentrations by sampling site ranged from 0.19 to 1.85 EU/m^3, and all endotoxin concentrations measured in this study were < 5.5 EU/m^3. This is lower than recognized occupational thresholds for acute or chronic adverse health effects previously reported (Castellan et al. 1987; Donham et al. 1989; Milton et al. 1996; Rylander 1997; Zock et al. 1998). These levels ranged from 40 to 1,000 EU/m^3 depending on the health outcome (pulmonary function changes, systemic effects, or airway inflammation), characteristics of the exposed population, and the methods of endotoxin exposure analysis employed. Zock et al. (1998) evaluated exposure–response data from 61 male potato-processing workers and found evidence of acute airway obstruction for 8-hr exposures to concentrations > 53 EU/m^3. If this exposure level was adjusted to deliver a comparable dose over a 24-hr day, this would correspond to a threshold ambient concentration of 17 EU/m^3, 38-fold higher than the mean value measured in the present study.

Exposure–response data have also been reported from human exposure studies. Kline et al. (1999) exposed 72 healthy, nonasthmatic, nonsmoking subjects to increasing doses of endotoxin via an inhalation-actuated nebulizer. Each dose was administered over a 20-min period, and spirometry was performed after each dose. Subjects were identified as sensitive, intermediate, and low responders, based on the amount of endotoxin required to induce a 20% decline in FEV_1 (forced expiratory volume in 1 sec). The eight sensitive subjects were disproportionately female (87%) and responded to the lowest trial dose (5,000 EU) with a significant drop in FEV_1. To achieve a comparable endotoxin dose through tidal breathing of ambient air over a 24-hr period would require an airborne concentration of 900 EU/m^3. This is also well above the concentrations measured in the present study.

Four of the five communities with the highest endotoxin concentrations were located in the downwind plume of Los Angeles [Rubidoux, Fontana, Perris, and UC (University of California) Riverside]. The site with the second highest concentrations of endotoxin, Lancaster, is situated at the western edge of the Mojave Desert at an elevation of 760 m. The Lancaster air parcel is the result of competitive wind pattern flows from the central California agricultural areas in the San Joaquin Valley and leakage from the Los Angeles suburbs (San Fernando Valley) through the lower passes east of Los Angeles and near the eastern edge of the LA Basin (San Bernardino). Thus, the most likely source of the endotoxin is the agricultural activities in the San Joaquin Valley. No obvious local source of endotoxin (e.g., agriculture, composting, waste treatment, cooling towers) was identified in the vicinity of the sampling station.

The highest endotoxin levels measured in this study were in Rubidoux, a community in close proximity to dairy farms with a census of > 15,000 cows. This association of elevated endotoxin with agriculture has been previously reported (Thorne et al. 2001, 2003b). We recently measured endotoxin concentrations in rural Iowa over a 15-month period 30 m and 160 m downwind of animal feeding operations housing swine. The geometric mean values (and geometric SDs) were 95.5 (2.95) EU/m^3 at the near site and 30.7 (2.0) EU/m^3 at the far site. Values for sites 30 m upwind were 9.3 (5.7) EU/m^3, whereas values in the barns were 3,100 (5.8) EU/m^3.

The concentration of endotoxin in the PM in this study ranged from 0.7 to 96.8 EU/mg. This is comparable with values from indoor settled dust but not with values downwind of swine barns. Data from the National Survey of Endotoxin in Housing reveal a 5th to 95th percentile range from 6.9 to 297 EU/mg for 2,469 samples collected from 790 homes across the United States (Thorne et al. 2003a). In contrast, airborne inhalable dust 30 m downwind of Iowa swine barns averaged 360 EU/mg in concentration, whereas upwind samples from these barns averaged 64.8 EU/mg (Thorne et al. 2001).

Although the ambient endotoxin concentrations found in this study are below no-effect levels found from occupational studies, concentrations are comparable with those measured in indoor samples where associated health effects have been reported. Therefore, it is possible that the low concentrations of endotoxin measured in this study may still be significant, especially in conjunction with other components of urban air pollution. Furthermore, the effect of outdoor endotoxin on indoor levels has not been well described. Most studies of endotoxin in the indoor environment rely on measurements of endotoxin in settled household dust. This may not reflect indoor airborne endotoxin concentrations but provides a useful means for classification of subjects by endotoxin exposure in studies of childhood asthma. In a 14-month study of 20 homes of employees of Harvard School of Public Health in the Boston, Massachusetts, area, Park et al. (2000) reported indoor airborne endotoxin concentrations ranging from 0.02 to 19.8 EU/m^3. Concentrations were highest in the spring and lowest in the winter but were not well correlated with endotoxin concentrations in settled dust. When compared with weekly or bimonthly outdoor concentrations in total suspended particulate, indoor concentrations were significantly higher in the winter but similar to outdoor concentrations during the rest of the year. The authors concluded that outdoor endotoxin may influence indoor concentrations during the warm weather months.

We were concerned that we might underestimate endotoxin concentrations in the air samples because of the centrifugation step in our filter extraction protocol. Upon placing the filters from the downwind plume of Los Angeles into the elution medium (pyrogen-free water with 0.05% Tween-20), the solution turned deep gray to black with the OD exceeding that tolerable even in a kinetic chromogenic assay. This color change was apparently caused by suspended soot particles and was effectively eliminated through centrifugation as shown in Figure 2. If endotoxin molecules were tightly bound to soot particles in such a manner that they could be lost in centrifuging the filter eluate but could react with lung cells if inhaled, we could underestimate biologically relevant exposure. To address this concern, we included 0.05% Tween-20, a non-ionic surfactant, in the filter extraction medium and in the dilution solution and vigorously shook the samples in this extraction medium for 60 min to maximize the solubilization of endotoxin. To test the effectiveness of this extraction method, we performed spiking assays in which PM_{10}-laden air sampling filters were spiked with endotoxin, dried, and then extracted and assayed. Complete recovery of the spiked endotoxin showed there was minimal loss of endotoxin via the soot particles.

Determination of endotoxin in environmental samples has been reported repeatedly in the literature, although the vast majority of studies have focused on air samples from occupational settings and settled dust samples collected in homes (reviewed by Heederik et al. 2003). To our knowledge, no previous studies have sought to optimize methods for determination of endotoxin concentrations in PM_{10} samples. Using air samples from occupational environments, Douwes et al. (1995) demonstrated that the use of 0.05% Tween-20 in the elution medium markedly enhances endotoxin extraction efficiency. Reasons proposed for this enhancement included *a*) disruption of

hydrophobic interactions between LPS and filter material, *b*) release of cell-wall–bound endotoxin, and *c*) dissociation of endotoxin micelles.

Two reports from one group of researchers have suggested that endotoxin in residual oil fly ash and concentrated air particles may not be readily detectable in the supernatant of extracts (Imrich et al. 2000; Ning et al. 2000). Their evidence was based on production of inflammatory cytokines from cell cultures with and without treatment of leachates with polymyxin B. However, these experiments did not establish that the cytokine release *in vitro* was due to endotoxin adsorbed on the particles. It is well established that many airborne contaminants besides endotoxin induce production of inflammatory cytokines. We previously reported that grain dust extracts treated with polymyxin B to reduce endotoxin retained much of their inflammatory potency as measured by *in vivo* cytokine production and airway neutrophilia (Jagielo et al. 1996). It is also noteworthy that Ning et al. (2000) and Imrich et al. (2000) used saline without any surfactant in the extraction process; thus, their results may not translate to our study.

This article provides the first evidence that urban air pollution contains relatively modest concentrations of endotoxin, even in areas with high PM_{10}. Additional studies are needed to further characterize outdoor endotoxin variations due to geographical and climatic factors. Furthermore, although the health effects of indoor exposure to low-levels of endotoxin have been investigated, further research is needed to determine what role endotoxin in outdoor air plays in respiratory conditions, both alone and in combination with other pollutants.

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