Biologic Effects Induced *in Vitro* by PM_{10} from Three Different Zones of Mexico City

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Exposure to urban airborne particles is associated with an increase in morbidity and mortality. There is little experimental evidence of the mechanisms involved and the role of particle composition. We assessed cytotoxicity (crystal violet assay), apoptosis [terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) or annexin V assay], DNA breakage (comet assay), and production of proinflammatory mediators [tumor necrosis factor α (TNFα), interleukin 6 (IL-6), prostaglandin E_2 (PGE_2)] (enzyme-linked immunosorbent assay), and E-selectin (flow cytometry) in cell lines exposed to particulate matter < 10 μm in size (PM_{10}) obtained from the northern, central, and southern zones of Mexico City. Particle concentrations ranged from 2.5 to 160 μg/cm^2. We used epithelial, endothelial, fibroblastic, and monocytic cells and assessed DNA damage in Balb-c cells, TNFα and IL-6 production in mouse monocytes, and PGE_2 in rat lung fibroblasts. We determined the expression of E-selectin in human endothelial cells and evaluated the cytotoxic potential of the PM_{10} samples in all cell types. PM_{10} from all three zones of Mexico City caused cell death, DNA breakage, and apoptosis, with particles from the north and central zones being the most toxic. All of these PM_{10} samples induced secretion of proinflammatory molecules, and particles from the central zone were the most potent. Endothelial cells exposed to PM_{10} from the three zones expressed similar E-selectin levels. Mexico City PM_{10} induced biologic effects dependent on the zone of origin, which could be caused by differences in the mixture or size distribution within particle samples. Our data suggest that particle composition as well as particle size should be considered in assessing the adverse effects of airborne particulate pollution. *Key words:* **apoptosis, cytotoxicity, DNA breakage, E-selectin, IL-6, Mexico City, particle composition, PGE_2, PM_{10}, TNFα.** *Environ Health Perspect* **110:715–720 (2002). [Online 4 June 2002]**
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Daily exposure to airborne particulate pollution in urban zones is associated with an increase in morbidity and mortality, mainly of cardiopulmonary origin (*1*). The increase in health risk occurs even when particle concentrations are well below established air quality standard levels. Epidemiologic associations of particles of smaller aerodynamic size with mortality are even more significant. For example, the increase in death risk reported for Mexico City with respect to total suspended particles (TSP) is 5.8% in mortality per 100 μg/m^3 TSP increment (*2*). However, particles with aerodynamic diameters of 10 μm and less (PM_{10}) or 2.5 μm and less ($PM_{2.5}$) cause a 1.83% or 1.48% increment in risk of death per 10 μg/m^3 increase of particulate matter (PM) (*3,4*). Therefore, PM size is clearly an important factor in the health risk of air pollution in Mexico City. Similar epidemiologic findings relating smaller PM diameter to increased mortality have been reported in several cities around the world, despite a wide variation in geographical location, climate, or economic development (*1*).

Particle composition may also play a major role in defining the relative toxicity of an inhaled particulate. However, although air pollution PM has been implicated in the development of several pulmonary diseases (asthma, chronic bronchitis, lung cancer), we currently have little information to define the role of particle composition to the progression of these respiratory diseases (*5–8*). Growing evidence suggests that several factors in air pollution PM could play a role in promoting and perpetuating a lung inflammatory or carcinogenic response. For example, several metals detected in Mexico City PM_{10}, including vanadium, nickel, and lead, are known to cause inflammation or cancer (*9*). These same metals have been reported to mediate the toxic effects of fly ash particles generated during the industrial burning of coal and fuel oil (*10*). Organic components may also play a role in mediating the inflammation caused by the inhalation of PM. Lipopolysaccharide (LPS) derived from the walls of gram-negative bacteria has been considered a major factor in the exacerbation and development of asthma (*11,12*). We have reported that endotoxin is an important constituent of Mexico City PM_{10} that triggers the production of the proinflammatory cytokine interleukin 1β (IL-1β) by rat pulmonary macrophages *in vitro* (*9*). Hydrocarbons present in particles from diesel exhaust have been reported to increase the production of total allergic antibody [immunoglobulin E (IgE)] in nasally challenged individuals, and IgE is known to mediate allergic lung inflammation (*13*). Therefore, we have evidence that both organic and inorganic components mediate the toxic effects of air pollution PM.

In this study, we evaluated PM_{10} from three different zones of Mexico City (northern, central, southern) on a variety of different cell types for their potential in triggering a variety of inflammatory and toxic end points in cultured cells *in vitro*. The northern zone is the major industrial center of the city, and particles collected from the northern zone contain higher levels of transition metals and sulfur (*9*). PM_{10} from the southern zone contains a higher level of organic material (e.g., pollen) (*6*). Automotive emissions, including hydrocarbons from diesel, contribute heavily to the PM burden in all three zones (*14*). The purpose of the present study was to assess comparatively the toxic and inflammatory potential of PM_{10} samples from the three zones of Mexico City and relate these findings to the particular particle mixture from each zone.

Materials and Methods

Reagents. We used the following: Apoptosis detection by the terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) method (Roche, Mannheim, Germany), apoptosis detection by the annexin V method (Nexins Research,

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Kattendijke, The Netherlands), enzyme-linked immunosorbent assay (ELISA) for tumor necrosis factor α (TNFα) and IL-6 (R&D Systems, Minneapolis, MN, USA) and for prostaglandin E_2 (PGE_2; Endogen, Woburn, MA, USA). We also used basic fibroblast growth factor and epidermal growth factor (Prepro Tech, London, England). Sera used were bovine fetal serum (Harlan, Indianapolis, IN, USA) and goat fetal serum (Sigma, St. Louis, MO, USA). Antibodies were murine monoclonal antibody to human E-selectin (CD62E; R&D Systems), mouse fluorescein isothiocyanate (FITC)-conjugated anti-IgG (Sigma).

Cells. We used the commercial cell lines A549 (human lung epithelium cells), J774A.1 (mouse monocytes), and Balb-c (mouse fibroblasts). All cell lines were from American Type Culture Collection (Manassas, VA, USA). Other primary cell cultures used were rat lung fibroblasts (RLF) and human umbilical vein endothelial cells (HUVEC), obtained from the National Institute of Environmental Health Sciences and Nottingham University, respectively.

PM sampling. We collected PM_{10} in the northern (industrial), central (business), and southern (residential) zones of Mexico City (Figure 1) using a high-volume particle collector (GMW model 1200 VFC HVPM10; Sierra Andersen, Smyrna, GA, USA) for particles with aerodynamic diameter ≤ 10 μm. We used fiberglass filters (type A/E glass 61638; Gelman Sciences, Ann Arbor, MI, USA) for 24-hour sampling (1.13 m^3/min), 3 days during each week of 1991 and obtained a total of 210, 211, and 203 samples from the south, center, and north, respectively. To obtain homogeneous samples throughout the year, we selected a filter each week from each zone to recover particles. We dry sonicated filters for 45 min and recovered particles by smooth sweeping with a brush into an endotoxin-free flask. We mixed the particles of a single year by zone and stored them dry in endotoxin-free glass vials in a dryer at 4°C until their use. We determined metal and endotoxin levels in these samples, which we have reported elsewhere (*9*). We prepared suspensions of PM (1 mg/mL) immediately before cell exposure to minimize dissolution of particle components. We used particle concentrations in μg/cm^2 as a better indicator of exposure to cells that attach and spread on the surface of culture wells, as previously done by our group (*9,16*). We took care to control the volume with which we added particles to cultures and always maintained an equivalence of 1 μg/cm^2 equals 2 μg/mL.

Cytotoxicity. We measured cytotoxicity using the crystal violet method (*17*). To assess cytotoxicity, we exposed proliferating or confluent cultures of Balb-c, J774A.1, A549, or RLF cells to particle concentrations of 10, 20, 40, 80, and 160 μg/cm^2 of PM sample. For the proliferating cultures, we seeded cells in 96-well plates at a density of 15,000 cells/cm^2 to determine cytotoxicity after 24, 48, and 72 hr of exposure. For the confluent cultures, we seeded cells in 96-well plates at a density of 180,000 cells/cm^2 and, once they were confluent, exposed them to PM for 24 hr in serum-free culture medium. We determined cytotoxicity by measuring the residual cell number with crystal violet staining with an ELISA plate reader at 595 nm (Multiscan MS 352; LabSystems, Helsinki, Finland). We calculated percentage viability comparing the absorbance of exposed cultures with the absorbance of nonexposed cultures.

Apoptosis assays. We measured apoptosis in A549 and J774A.1 using the TUNEL assay (*18*) and in HUVEC using fluoresceinated annexin V for phosphatydil serine detection (*19*), and performed the analysis by flow cytometry (Scan Flow; Becton-Dickinson, San Jose, CA, USA). We plated cells on 6-well dishes at a density of 15,000 cells/cm^2 in 1% fetal bovine serum (FBS)–Dulbecco's modified Eagle's medium (DMEM) and antibiotics. After 24 hr, we changed the medium for complete medium (10% FBS–DMEM + antibiotics) and added particles from the three regions at 160 μg/cm^2. We harvested cells after 24 hr and performed TUNEL or annexin V assays following the manufacturer's instructions. We assessed 10,000 cells by flow cytometry and determined the percentage of marked cells, which we considered apoptotic cells.

Comet assay for DNA breakage. We determined the ability of PM_{10} to cause DNA breakage by agarose gel cellular electrophoresis following a method previously described (*20*). We plated cells 20,000/well and exposed to concentrations of 2.5, 5, 10, 20, and 40 μg/cm^2 of PM_{10}. After 72 hr of exposure, we trypsinized cells (0.05%), mixed 10,000 cells in 1% low-fusion-point agarose, and then placed the mixture on a slide covered with previously solidified agarose. We treated the sample with digestion buffer [10 mM Tris, 100 mM ethylenediamine-tetraacetic acid (EDTA), 2 M NaCl, 1% Triton X-100, pH 10] for 30 min, then rinsed it in a solution of 100 mM NaCl, 1 mM EDTA and subjected it to 50-V electrophoresis for 30 min (Horizon 58; Gibco-BRL, Gaithersburg, MD, USA). We rinsed the sample in cold buffer for 5 min and added 10 μL of 1 μg/mL ethidium bromide. We randomly analyzed 30 cells per slide under fluorescence microscopy (BX40F; Olympus, Tokyo, Japan) after capturing the image with a video camera (TK-1280E, JVC). We measured the length of each comet using an image analysis system (PC Image color/Windows 3.1; Foster Findlay, Newcastle, UK).

Cytokine assays. We exposed confluent cells cultured in 24-well plates to increasing concentrations of PM_{10} (10, 20, 40, and 80 μg/cm^2) in 1 mL of DMEM. After 24 hr, we collected cell supernatants, centrifuged them at 14,000 × *g* for 15 min, and frozen them at –70°C. We used nonexposed cells as negative control and cells exposed to 10 μg/mL LPS from *Escherichia coli* 055:B5 (Sigma) as positive control. We measured TNFα and IL-6 in the supernatants of J774A.1 cells and PGE_2 in supernatants from RLF using commercial kits following the manufacturer's instructions. We express these results as a percentage of the positive controls or in pg/mL.

E-selectin expression. We evaluated E-selectin expression as previously described (*21*). We plated HUVEC on 25 cm^2 flasks until they reached confluency and then exposed them to 40 μg/cm^2 of PM_{10}. We used TNFα (10 ng/mL) as a positive control and unexposed cells as negative control. After 6 hr of exposure, we harvested cells with 10 mM EDTA in phosphate-buffered saline (PBS). We resuspended cells in 20% goat fetal serum–PBS for 20 min at 4°C, rinsed them in PBS, and then incubated them with anti–E-selectin antibody (10 μg/mL) for 1 hr at 4°C, and rinsed and resuspended them in FITC-murine anti-IgG

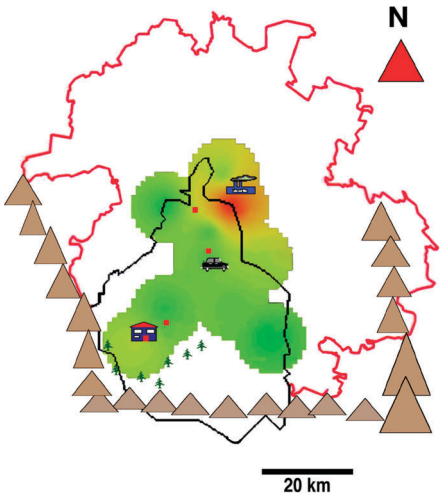

Figure 1. Map of Mexico City. The metropolitan area is composed of two regions: the Federal District (black outline) and the Estado de Mexico (red outline). Air quality monitoring stations cover the area shown in green. The most polluted areas for particles and ozone are in the northeast (red) and the southwest (yellow), respectively. We collected particles for this study in the northern (industrial), central (business), and southern (residential) zones; sites of collection are marked with red squares. Mountains (brown triangles) surround the city, mainly in the south. Adapted from Gobierno del Distrito Federal (*15*).

antibody (10 µg/mL) for 30 min at 4°C in the dark. We fixed cells with 4% paraformaldehyde in PBS and kept them in the dark at 4°C until analysis by flow cytometry. We measured the presence of E-selectin in 10,000 cells as fluorescence intensity after subtracting the fluorescence values obtained from cells that we incubated in the absence of the primary antibody.

Statistical analysis. We repeated all experiments at least three times and express results as mean ± standard deviation. We present results from the comet assay as median, maximum, minimum, 25th and 75th percentiles, and outlying values, and express TNFα, IL-6, and PGE_2 protein levels (pg/mL) as a percentage of the values obtained from the positive controls.

We evaluated cytotoxicity, DNA breakage, and cytokine production (TNFα, IL-6, PGE_2) results by two-way analysis of variance (ANOVA), and apoptosis and E-selectin by one-way ANOVA. We performed all analyses with Intercooled Stata for Windows (version 6.0, 1999; Stata Corp., College Station, TX, USA), and we considered differences significant when $p < 0.05$.

Results

Table 1 presents PM_{10} levels present in the three zones of the study during 1991.

Cytotoxicity. PM produced a concentration-dependent cytotoxic effect that was apparent after 72 hr in proliferating cells of monocytic and fibroblastic origin (Figure 2). Particles from the northern zone showed a statistically significant larger effect than did particles from the central and southern zones ($p < 0.0001$). Cell types differed in viability. Monocytic cells were more susceptible, reaching a 70% loss of viability with 160 µg/cm^2 of particles from the north and ~50% with particles from central or south zone ($p < 0.0001$; Figure 2A). Fibroblastic cells were less susceptible: 160 µg/cm^2 of particles from the north induced 65% loss of viability and only ~20% with particles from the central or southern zone ($p < 0.0001$; Figure 2B). Epithelial cells were the most resistant to PM, with little or no loss in viability over PM concentration ranges (Figure 2C).

Particles were not cytotoxic in confluent, quiescent cultures of any of the cell types that we exposed to 20–160 µg/cm^2 of the PM_{10}. Resistance to cytotoxic effects occurred regardless of the zone of origin of the particles (data not shown).

Apoptosis. Particles induced low ratios of cell death by apoptosis. In J774A.1 cells, particles from the three zones induced similar apoptosis levels, which were ~15% (Figure 3A). In A549 cells, the apoptosis indexes

Table 1. PM_{10} levels (µg/m^3) in three different zones of Mexico City, 1991.

	Northern zone	Central zone	Southern zone
Mean ± SE	121.6 ± 4.08	107.2 ± 3.28	75.7 ± 2.21
Range	372.5–22.9	329.6–39.7	182.7–20.0
Lower quartile	79.6	72.0	52.0
Upper quartile	158.1	138.0	96.3

See Bonner et al. (*9*) for transitional metal and endotoxin content of these samples.

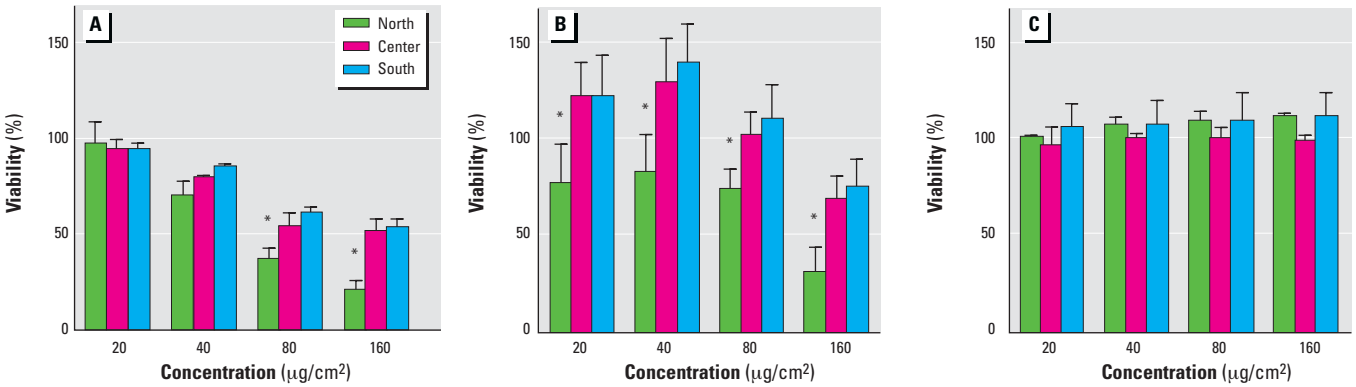

Figure 2. Viability of proliferating cells after 72 hr exposure to PM_{10} from three Mexico City zones. (*A*) J774A.1 cells were the most susceptible and showed dose-related effects. Particles from the northern zone showed the greatest effect ($p < 0.0001$). (*B*) Balb-c cells also responded in a dose-related manner, with particles from the northern zone being the most cytotoxic ($p < 0.0001$). (*C*) A549 cells were not affected by any particle concentration. Viability was determined by the crystal violet colorimetric method. Values are mean + SD.
*Statistically significant differences.

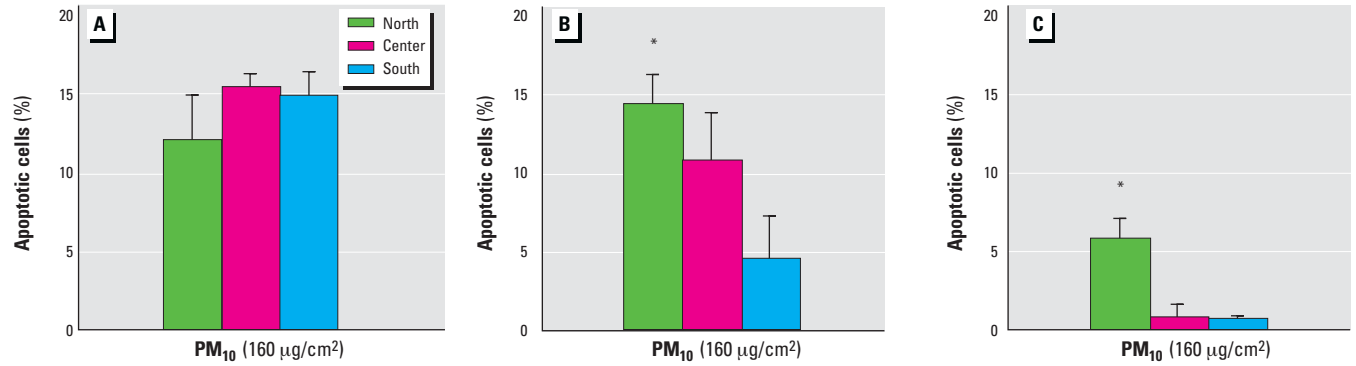

Figure 3. Percentages of apoptosis induced in cell cultures exposed to 160 µg/cm^2 of PM_{10} from three different Mexico City zones. (*A*) J774A.1 cells responded equally to particles from the three regions. (*B*) A549 cells responded in a gradient pattern from north to south ($p = 0.004$). (*C*) HUVEC were affected only by particles from the north ($p = 0.0015$). We used TUNEL and annexin V assays for these experiments, as described in "Materials and Methods." Values are mean + SD.
*Statistically significant differences.

were 4% for the south, 11% for the center, and 15% for the north (p = 0.004; Figure 3B). HUVEC showed increased apoptosis only when exposed to particles from the north (6 ± 1.5%; p = 0.0015; Figure 3C).

DNA breakage (comets). All PM_{10} from Mexico City were able to induce DNA breakage measured as an increase in the electrophoretic mobility of nuclear material (comet) of exposed cells. We observed dose–response effects with concentrations between 2.5 and 10 μg/cm^2 (p < 0.0001; Figure 4). Northern and central zone particles produced longer comets, compared with those of the southern zone (p < 0.0001). More than 50% of the comets had a length above the 75th percentile from control cells, with 2.5 μg/cm^2 PM_{10} from the northern or the central zone. On the other hand, particles from the southern zone required 10 μg/cm^2 to induce a similar effect. At concentrations of 20 and 40 μg/cm^2, comet length did not increase beyond that obtained with 10 μg/cm^2 (results not shown).

Presence of TNFα and IL-6 in supernatants of J774A.1 cells. Particles induced TNFα and IL-6 secretion in this monocytic cell line in a dose-dependent manner, and maximal production was attained with 80 μg/cm^2 (p < 0.0001). TNFα production had a trend suggesting that particles from the central zone were more powerful than were particles from northern and southern zones, but we found no statistical significance (Figure 5A). IL-6 secretion also showed the same trend, but in this case differences were statistically significant (p < 0.0001; Figure 5B). Maximal TNFα and IL-6 average secretion observed with particles from the center was 37.7 ± 8.4% and 7.1 ± 0.3%, respectively. We express these results as percentages of levels induced by the positive control of each experiment. Negative control unexposed cells secreted 129 ± 116 pg/mL (0.85 ± 0.81%) of TNFα, whereas cells stimulated with LPS (10 μg/mL) reached levels of 17,817 ± 4,876 pg/mL (100%). We did not detect IL-6 in the supernatant of nonexposed cells, whereas cells stimulated with 10 μg/mL LPS secreted 2,356 ± 64 pg/mL (100%) IL-6.

PGE_2 production by RLF. PM_{10} induced PGE_2 secretion in RLFs in a dose-related manner (p < 0.0001; Figure 6). Particles from the central zone always induced the most PGE_2 secretion (p = 0.0021). We obtained maximal secretion at 40 μg/cm^2 with particles from the three zones, reaching 69 ± 6%, 163 ± 28%, and 91 ± 2% for the northern, central, and southern zones, respectively. We express results as percentages of levels induced by the positive control of each experiment. Cultures unexposed to particles secreted 60 ± 1 pg/mL (5%), and those exposed to 10 μg/mL LPS, 1,273 ± 13 pg/mL (100%).

Expression of E-selectin in HUVEC. Endothelial cells had a 25% increase in E-selectin expression after exposure to 40 μg/cm^2 of PM_{10} from the three zones of Mexico City (Figure 7).

Discussion

In this article we present data indicating that PM_{10} from Mexico City is able to induce *in vitro* cell death, DNA damage, and the production of TNFα, IL-6, and PGE_2 in a differential pattern related to the site in the city where we collected particles (Table 2). PM induced these effects in different cell types in a dose-related manner, providing biologic plausibility to epidemiologic studies.

Cell death and apoptosis have been used as markers of cell injury induced by urban

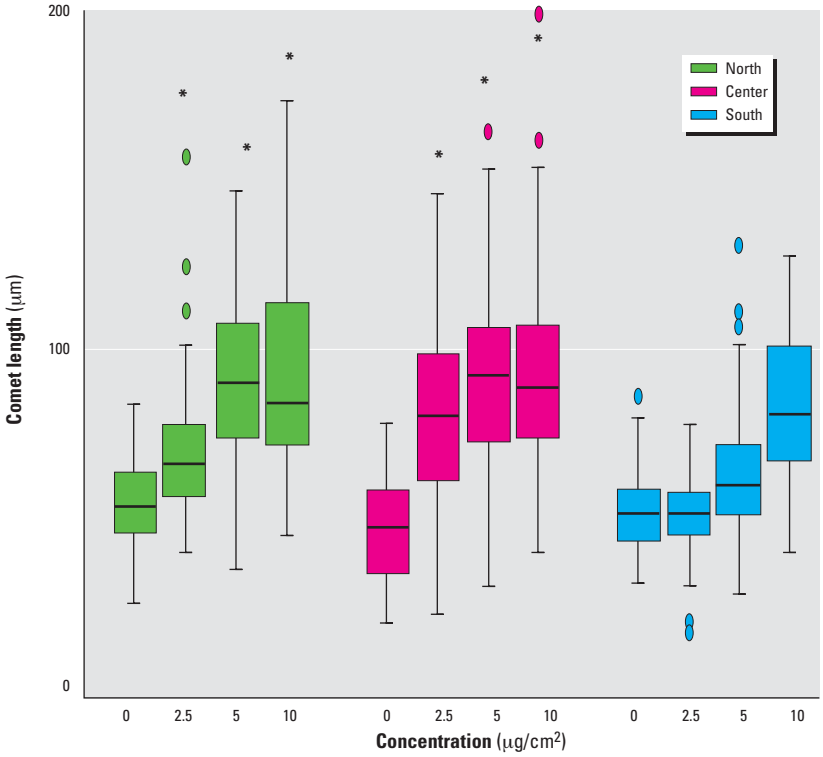

Figure 4. Distribution of comet length in Balb-c cells exposed to PM_{10} from three Mexico City zones. All samples induced in a dose-related response (p < 0.0001). Particles from the northern and central zones induced longer comets than did particles from the south when we used 2.5 or 5 μg/cm^2 (p < 0.0001). Values are the median, 25th and 75th percentiles, and maximum and minimum values. The ovals represent outlying values. Values presented under the boxes from the center represent the concentrations (μg/cm^2) used in these experiments and apply to all three regions.
*Statistically significant differences.

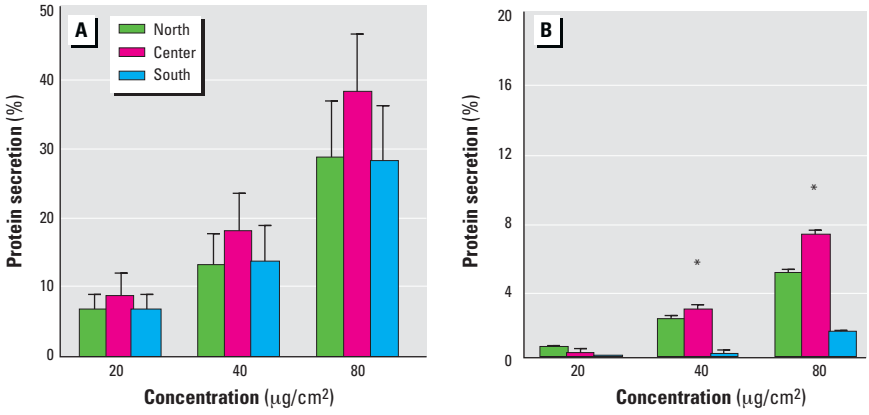

Figure 5. Particles from three different Mexico City zones induced the secretion of TNFα (*A*) and IL-6 (*B*) in a dose-related manner in J774A.1 cell cultures exposed to 20, 40, and 80 μg/cm^2 PM_{10}. Particles from the central zone had a tendency to be more potent, which was statistically significant for IL-6 (p < 0.0001). Results are expressed as a percentage of the values obtained from cells stimulated with 10 μg/mL LPS. Error bars indicate SD.
*Statistically significant differences.

particles (*22–24*) and most recently have been proposed as mediators in asthma exacerbation induced by PM (*25*). In this study, we observed cell death only in proliferating cells, indicating the existence of vulnerable points during the cell cycle. Similar results have been reported for cells exposed to asbestos, but the significance of this phenomenon remains unknown (*16*). Some of the cell lines studied are more susceptible than others. Epithelial cells are the most resistant to particle-induced cell death. In the case of susceptible (monocytic and fibroblastic) cells, particles from the northern zone of Mexico City had a greater cytotoxic effect. A previous study indicated that particles from two different U.S. cities induced cell death (*22,24*) and apoptosis (*24*) in human alveolar macrophages at similar levels. However, the sources and type of pollution in them were very similar and did not show the contrasts described for Mexico City particles. Sublethal doses of particles from Mexico City produced DNA damage in Balb-c cells (a common cell type used for DNA damage assays) (*26*). The participation of apoptosis in the cell death process was relatively small, suggesting that DNA damage could be repaired. Cell death, apoptosis, and DNA damage were more apparent for particles from the northern and central zones. This kind of effect needs further study as a possible mediator of cancer related to air pollution.

Inflammatory mediators, including cytokines (e.g., TNFα and IL-6) and prostaglandins (e.g., PGE_2), play major roles in pulmonary inflammation related to particle pollution (*23,27,28*). PM_{10} from Mexico City was able to induce the production of inflammatory mediators in various cell types. We evaluated this in cell cultures using particle concentrations that did not affect cell viability. Cells of monocytic origin produced increased levels TNFα and IL-6 after particle stimulation. Particles from the central zone of Mexico City were the most potent in causing cytokine secretion. We also observed a similar pattern for PGE_2 production by fibroblasts. In endothelial cells exposed to PM_{10}, we observed an increase in the presence of E-selectin, a molecule that plays an important role in recruiting circulating leukocytes during inflammation (*29*). In this case, we found no differential effects by zones. Collectively, these data suggest that TNFα, IL-6, and PGE_2 could be important in mediating the inflammatory effects of Mexico City PM in relation to the zone where we collected the particles. Other authors have also shown that particles from different cities induce the secretion *in vitro* of proinflammatory molecules but made no attempt to discuss differences found among cities (*22*).

Interestingly, studies showing *in vitro* cellular effects induced by PM collected in different cities of the world found such effects with concentrations within the range we used in this study, despite the fact that we collected particles and handled them in different ways. Unfortunately, we have no instrument that allows collecting particles in large amounts without the introduction of artifacts such as loss of volatiles (*9*) or soluble components (*24,30*), trapping of particles in the matrix of the collection substrate (*9*; present results), the introduction of solvents to dislodge particles from the collecting substrate (*14,23*), introduction of contaminants from the collecting substrate (*31*), or differences in the particle aerodynamic diameter range used (*9,23,24,28*). Therefore, comparisons between studies are difficult, and these limitations cannot be overcome until a standard method for collection exists, which should be kept in mind.

Differences in the metal and endotoxin contents previously reported for these particles from three Mexico City zones (*9*) may account for the variations in effects evaluated in this work. Metal content showed a decreasing north-to-south gradient, whereas endotoxin showed similar concentrations in the three zones. The relative importance of metals and endotoxin in mediating the toxic effects of these particles is currently unknown (*9,10,23,28*). In the present case, metal content may be related to the toxic effects produced by particles from the northern zone, but less directly to the proinflammatory effect observed with central zone particles. Particles from the northern zone showed a trend to induce cell death and DNA damage in proliferating cells, whereas those from the central zone induced proinflammatory responses. The fact that this proinflammatory effect became more noticeable when we used particles with less metal content suggests that endotoxin effects might then be seen, given that a previous study showed that biologic effects induced by these particles may be results of synergisms between metals and endotoxin (*9*). The role of endotoxin present in these samples is currently under study in our laboratory. However, our knowledge about the particle composition is still limited, and we cannot eliminate the role of other components.

At the present time, the toxicity of air pollution particles has been largely linked to aerodynamic diameter and their capability to reach the lower respiratory tract, providing a large surface area to interact with the target cells. However, our results provide new insight to the hypothesis that particle composition can also play a role. Recent epidemiologic evidence points in that direction and indicates that among $PM_{2.5}$ components, elemental carbon, organic carbon, and potassium from burning vegetation had a

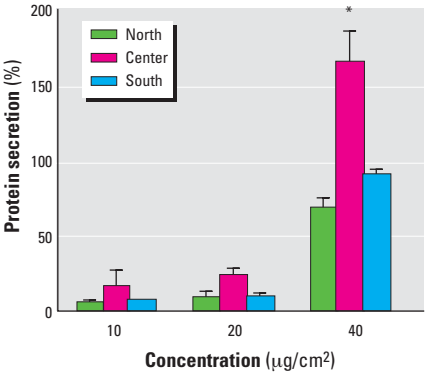

Figure 6. Production of PGE_2 by RLFs stimulated with PM_{10} from three regions of Mexico City occurred in a dose-related manner ($p < 0.0001$). Particles from the central zone induced higher PGE_2 levels at all concentrations ($p = 0.0021$). Results are expressed as a percentage of the values obtained from cells stimulated with 10 μg/mL LPS. Error bars indicate SD.
*Statistically significant differences.

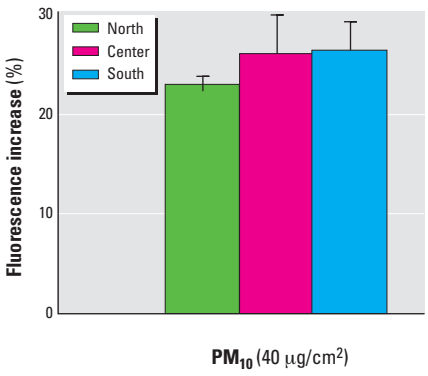

Figure 7. Expression of E-selectin by endothelial cells was stimulated around 25% when incubated 6 hr with 40 μg/cm² PM_{10}. We observed no difference among regions. Error bars indicate SD.

Table 2. Semiquantitative comparative appreciation of cellular effects induced by PM_{10} from Mexico City.

	Cytotoxic effects			Proinflammatory effects			
Zone	Toxicity	Apoptosis	DNA damage	TNFα	IL-6	PGE_2	E-selectin
Northern	+++	+++	+++	++	++	+	++
Central	++	++	+++	+++	+++	+++	++
Southern	++	+	++	+	+	++	++

The number of + symbols indicates the magnitude of the observed effects.

positive association with cardiovascular mortality in the elderly (*32*).

The clear epidemiologic evidence indicating an association between particle air pollution, acute adverse health effects, and increased mortality has led to hypotheses of pulmonary inflammation (*7*), increased blood viscosity (*27*), and alterations on heart rate variability (*33*) as possible mechanisms through which air pollutants could trigger adverse cardiopulmonary outcomes. We have experimental evidence to support each of these hypotheses (*23,28,34*), yet we are far from understanding the pathogenic mechanisms involved. The use of various cell types and end points could prove helpful in the future to explore pathogenic mechanisms for a wide range of diseases linked to particulate pollution, such as asthma, cardiovascular disease, and cancer.

Besides particle composition, we need to study particle size distribution in PM_{10} samples because particle effects could also be related to PM surface area, as suggested by Schwartz et el. (*35*). Studies done by others in Mexico City have shown that the $PM_{10}/PM_{2.5}$ ratios do not vary extensively among samples obtained in different regions of the city (*36,37*), giving support to the role of particle composition in the results presented in this study. Establishing more accurate relationships requires determining particle composition in different size fractions and understanding potential interactive effects among the different particle components. Studies in this matter are underway.

In conclusion, our results support the hypothesis that particle composition may account for the differences in the inflammatory and toxic responses induced by air pollution particles from three different zones of Mexico City. Particles from the northern zone of Mexico City that contained relatively high levels of transition metals were the most toxic in assays of cell death and DNA damage. However, particles from the central zone of the city were the most effective in causing the release of inflammatory mediators in cultured cells. Future research should identify the component or components that mediate, alone or in combination, the cytotoxic and proinflammatory potential of Mexico City air pollution particulates. The use of biologic response patterns, such as the ones presented here (Table 2), could prove useful indicators of biologic effects induced by complex pollutant particulate mixtures, instead of using single end points.

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