Aryl Hydrocarbon Receptor–Mediated Activity of Particulate Organic Matter from the Paso del Norte Airshed along the U.S.–Mexico Border

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In this study, we determined the biologic activity of dichloromethane-extracted particulate matter < 10 μm in aerodynamic diameter (PM_{10}) obtained from filters at three sites in the Paso del Norte airshed, which includes El Paso, Texas, USA; Juarez, Chihuahua, Mexico, and Sunland Park, New Mexico, USA. The extracts were rich in polycyclic aromatic hydrocarbons (PAHs) and had significant biologic activity, measured using two *in vitro* assay systems: ethoxyresorufin-*O*-deethylase (EROD) induction and the aryl hydrocarbon–receptor luciferase reporter system. In most cases, both EROD (5.25 pmol/min/mg protein) and luciferase activities (994 relative light units/mg) were highest in extracts from the Advance site located in an industrial neighborhood in Juarez. These values represented 58% and 55%, respectively, of induction associated with 1 μM β-naphthoflavone exposures. In contrast, little activity was observed at the Northeast Clinic site in El Paso, the reference site. In most cases, luciferase and EROD activity from extracts collected from the Tillman Health Center site, situated in downtown El Paso, fell between those observed at the other two sites. Overall, a statistically significant correlation existed between PM_{10} and EROD and luciferase activities. Chemical analysis of extracts collected from the Advance site demonstrated that concentrations of most PAHs were higher than those reported in most other metropolitan areas in the United States. Calculations made with these data suggest a cancer risk of 5–12 cases per 100,000 people. This risk estimate, as well as comparisons with the work of other investigators, raises concern regarding the potential for adverse health effects to the residents of this airshed. Further work is needed to understand the sources, exposure, and effects of PM_{10} and particulate organic material in the Paso del Norte airshed. *Key words:* air pollution, biomarkers, El Paso, particulate matter, PM, polycyclic aromatic hydrocarbons (PAHs), U.S.–Mexico border. *Environ Health Perspect* 111:1299–1305 (2003). doi:10.1289/ehp.6058 available via *http://dx.doi.org/* [Online 7 May 2003]

The Paso del Norte airshed is composed of a basin formed by mountains that surround El Paso, Texas, and Sunland Park, New Mexico, in the United States and Ciudád Juarez, Chihuahua, in Mexico. With a population exceeding two million, it is one of the largest metropolitan areas along the border. Visibility in the Paso del Norte airshed is frequently poor, especially in winter, and respiratory problems are common (Barron 1999). El Paso has been designated as a federal nonattainment area, associated with exceedances of ozone and particulate matter < 10 μm in aerodynamic diameter (PM_{10}). Recently, an index was developed to reflect long-term exposure to air pollutants (Kyle et al. 2002). U.S. cities were ranked according to a weighted estimate of exposure to criteria air pollutants; El Paso was ranked sixth worst in the nation, following Los Angeles, California; Phoenix, Arizona; Riverside, California; Orange County, California; and New York City, New York.

Sources of PM_{10} in the Paso del Norte airshed share some similarities with other urbanized areas, but some aspects of emissions and climatology are unique to this region. High ambient PM_{10} levels result from a wide range of emission sources, and their presence in air is affected by meteorologic conditions; in particular, strong inversions trap PM_{10} in the winter. Emissions are particularly high in the Paso del Norte airshed because of the high percentage of older vehicles, many without catalytic converters; a significant amount of diesel exhaust associated with North American Free Trade Agreement–related truck traffic at U.S.–Mexico border crossings; and the use of wood, tires, and other scrap fuels for both residential heating and the firing of bricks. These sources of PM_{10} are known to produce particulate organic material (POM) mixtures of thousands of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-*p*-dioxins, many of which are highly toxic and carcinogenic (Cuppitt et al. 1994; DeVito and Birnbaum 1995). Numerous epidemiologic studies have shown an association between ambient air particulates and increased morbidity and mortality (Folinsbee 1992; Pereria et al. 1998; Schwartz 1994). Recent studies have shown that ultrafine particles may play an important role in cardiopulmonary diseases (Pope et al. 2002). The mechanism by which PM causes these adverse effects is the topic of significant study (Harrison and Jianxin 2000). PAHs, including nitro-derivatives, account for approximately 80% of mutagenic activity in urban PM extracts (Alsberg et al. 1985; Harrison and Jianxin 2000). In addition to their mutagenicity, PAHs can interfere with certain developmental processes and nourishment of the fetus (Sram 1999). Environmental exposure to PAHs in heavily polluted areas such as Krakow, Poland, has caused increased levels of white blood cell PAH–DNA adducts in both mothers and infants. In this study, our goals were to identify the presence of these potentially harmful PAHs in POM by using two *in vitro* assays, to quantify levels of selected PAHs, and, to the degree possible, relate these findings to the risk of adverse health effects.

Materials and Methods

Site selection and sample collection. PM_{10} samples were collected on glass fiber filters using high-volume air samplers (1,400 m^3/24 hr) during the winter of 1998–1999 from two sites in El Paso and one site in Juarez. The sites selected in El Paso were Northeast Clinic, a site in a residential neighborhood about 1 mile from the desert, and Tillman Health Center, a site with high levels of PM_{10} in

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downtown El Paso. The Northeast site was selected as the reference site on the basis of gravimetric data indicating that PM_{10} levels are the lowest in the region. The site selected in Juarez was Advance Transformer, located next to a transformer production facility in an area of *maquiladoras* (foreign-owned or -operated assembly plants that import raw materials into Mexico and assemble finished products, primarily for export) and within 200 yards of a brick-making district. Brick makers typically use scrap fuels such as sawdust, tires, and lumber to fire the bricks. The El Paso City-County Health and Environmental District and Departemento de Ecología, Ciudád Juárez, were responsible for setting up the filters, collecting them after the 24-hr monitoring period, and recording gravimetric measurements. PM_{10} filters were then collected from the local agencies 1 week after the PM_{10} monitoring date. During this period, filters were protected from light and moisture. Using latex gloves, each filter was cut into a 5 × 8 in. rectangle with ethanol-rinsed scissors, wrapped in aluminum, labeled, and stored at –20°C in a dessicator until further processing.

Extraction of organic material. All glassware used for organic extractions and storage was washed with Alconox detergent (Alconox, Inc., White Plains, NY) for 20 min, rinsed with distilled water, and acid washed in 1 N nitric acid (ACS certified plus; Fisher Scientific, Pittsburgh, PA) for 1 hr. Glassware was then rinsed with distilled water, followed by rinsing with dichloromethane (DCM; Optima, Fisher Scientific) and baked for 1 hr at 100°C. One-half of an 8 × 10 in. filter was extracted individually using a Sohxlet apparatus with 100 mL of DCM for 24 hr, followed by concentration of the extract with a Kuderna-Danish apparatus according to U.S. Environmental Protection Agency (EPA) Method 3450C (Legzdins et al. 1995). DCM was used for extraction because it effectively extracts broad classes of organics, including those that are mutagenic, such as PAHs (Hannigan et al. 1994; Nielsen 1992). Samples were concentrated to a volume of approximately 1 mL and stored in amber vials with polytetrafluoroethylene-lined caps at 4°C. Before use of extracts for experiments, DCM was evaporated under a gentle stream of nitrogen in an N-VAP evaporator (Organomation, Berlin, MA) and the extracts were resuspended in dimethyl sulfoxide (DMSO; cell culture grade, Sigma, St. Louis, MO). In addition, glass fiber thimbles (Schleicher & Schuell, Keene, NH) and three clean PM_{10} glass fiber filters (5 × 8 in.) were extracted as described above and tested as controls/blanks using EROD and luciferase assays.

Cell culture. We used rat hepatoma (H4IIE) cells to measure CYP1A1-related enzyme activity [i.e., ethoxyresorufin *O*-deethylase (EROD)]. We purchased cells from the American Type Cell Culture (Marassas, VA) and cultured them in minimum essential medium (MEM), Eagle with Earle salts, containing 7.5% fetal bovine serum, 7.5% newborn calf serum, and 50 µg/mL gentamicin sulfate at pH 7.4. H4IIE cells were grown at 37°C in a humidified incubator with a 5% CO_2 atmosphere and subcultured weekly by dissociating with 0.25% (weight/volume) trypsin and 1 mM EDTA in calcium- and magnesium-free Hanks buffer, pH 7.4 (Sigma).

We used recombinant mouse hepatoma (H1L1.1c2) cells to determine the presence and relative activity of aryl hydrocarbon receptor (AhR) ligands in the chemical extract. H1L1.1c2 cells contain a stable integrated reporter plasmid (pGudLuc1.1) that responds to chemicals similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) with the induction of luciferase enzyme (Garrison et al. 1996). H1L1.1c2 cells were cultured in MEM α with L-glutamine, 10% fetal bovine serum, and 1% penicillin–streptomycin at pH 7.4, grown at 37°C in a humidified incubator with 5% CO_2, and subcultured biweekly.

Range finding. The selection of concentrations for cell exposure for experiments was based on an analysis of the response of both H4IIE and H1L1.c2 cells to various volumes of extracts. To identify physiologically relevant amounts of the DCM extracts to use, we estimated what an average adult male might be exposed to in a 24-hr period. We selected 20 m^3/day as the default for the ventilation rate for an adult male. Seventy percent of this total, or 14 m^3/day, was the estimate used for the alveolar ventilation rate, assuming 100% absorption. Finally, we used the default collection rate of 1,400 m^3/24 hr for the Hi-Vol PM_{10} sampler (Wedding & Associates Inc., Fort Collins, CO). To obtain the relationship between the volume of air (cubic meters) and the volume of DCM extract (microliters), we first divided 1,400 m^3 (volume of air through the Hi-Vol sampler in 24 hr) by 14 m^3 (alveolar air inhaled in 24 hr). This quotient was then divided by the total volume of DCM extract (microliters) for each sample. The dose range used for the EROD range finder was based on our estimate of the volume of air an adult male would breathe in 3–1,050 min, equal to 0.030–10.21 m^3. H4IIE cells were seeded at 450,000 cells/well and, after 24 hr, were incubated for an additional 24 hr with 15 µL (0.5% of total volume of cell culture media) of the DCM extract. After exposures, cells were rinsed with Hanks buffer and lysed with mammalian protein extraction reagent (M-PER, Pierce Chemicals, Rockford, IL). Homogenates were then centrifuged at 160 × *g* for 10 min at 4°C. Supernatants were collected and stored in 25 mM bicine buffer with 20% glycerol at –20°C.

We also tested the effects of various amounts of filter extracts for their ability to induce luciferase activity in H1L1.1c2 cells. In these experiments, cells were seeded at 150,000 cells/well and after growth overnight, cells were incubated with sample extracts for 4 hr with various amounts of DCM extracts, roughly equal to what an adult male might inhale in 1–515 min, or 0.068–5.0 m^3 of air. Extract volumes used were 0.5% of the total incubation volume in each well. After exposures, cells were rinsed with calcium- and magnesium-free phosphate-buffered saline and lysed with 100 µL lysis buffer (Promega, Madison, WI). Supernatants were collected and stored at –20°C.

Biochemical assays. We measured EROD activity fluorometrically, as previously described (Sanderson et al. 1996). In a 96-well Microlite-1 plate (Dynex, Chantilly, VA), we mixed 30 µL cell homogenates with 100 µL 50 mM HEPES buffer, pH 7.8, containing 40 µM dicumarol, and 50 µL of a 20 µM stock solution of ethoxyresorufin with 1.25% Tween 80. Wells were monitored for background activity for 5 min before the addition of 50 µL 0.5 mM NADPH, which was used to start the reaction. Excitation/emission wavelengths of 535/585 nm, respectively, were used to monitor EROD activity, which was expressed as picomoles per minute per milligram of protein. Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay (Pierce Chemicals) with bovine serum albumin as the standard.

We determined luciferase activity in 25 µL H1L1.1c2 homogenates in white opaque 96-well Cliniplates (Labsystems, Franklin, MA) followed by the addition of 100 µL luciferase assay reagent (Promega) as previously described (Garrison et al. 1996). Luciferase activity was quantitated for 10 sec, and activity was expressed as relative light units (RLUs) per minute per milligram of protein. The protein concentrations were also determined using the BCA assay (Pierce Chemicals).

To identify whether any of the extracts had the potential to produce overt toxicity in the cell lines, we used the XTT assay (Roehm et al. 1991). This assay measures the mitochondrial metabolism of the tetrazolium dye 2,3 bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide (Sigma). Cells were exposed to various concentrations of DCM extracts collected from the three PM_{10} sites along with various concentrations of DMSO (0.5, 1, 2%) or DMSO alone (negative control).

In vitro *bioassays.* Based on results of the range-finding studies, H4IIE cells were exposed to an amount of extract equivalent to 0.145 m^3 of air per well, whereas H1L1.1c2

cells were exposed to an amount equivalent to 0.0145 m^3 of air. Sample extracts for the months of December, January, and February from the Northeast, Tillman, and Advance sites were used for cell exposure studies. Twenty-four hours after subculturing in 6-well plates, DCM extracts were added to H4IIE cells for an additional 24 hr. Cells were then harvested, washed twice with Hanks buffer, and protein extracted with 30 µL M-PER. Cells were removed and centrifuged, and supernatant aliquots were stored at –20°C. EROD activity was measured as described above. H1L1.1c2 cells were cultured in 24-well plates overnight, seeded at a density of 150,000/well, and then exposed to DCM extracts for 4 hr; cells were washed and lysed as described above, and then immediately assayed for luciferase activity. Cells used for positive and negative controls were exposed to either 1 µM β-naphthoflavone (BNF) or DMSO, respectively. Both the EROD and luciferase assays were performed in quadruplicate.

Chemical analysis. Extracts from the Advance site were also analyzed for the concentration of selected PAHs. A small aliquot of each of the DCM extracts was diluted 10-fold (20 µL extract/200 µL DCM) so that the concentration of the target analyte fell within the linear range of the analytical method (~0.05–50 ng/µL). Samples were then spiked with a coinjection of standard (~0.1 ng/µL) that consisted of deuterated PAHs (chrysene-d_{12}–perylene-d_{12}; Z-014J; AccuStandard, Inc., New Haven, CT).

Samples were analyzed by the electron impact gas chromatography/mass spectrometry (GC/MS) technique, using a Hewlett-Packard 5890 gas chromatograph interfaced to a 5972 Mass Selective Detector operated in scan mode (Hewlett Packard, Wilmington, DE). One-microliter splitless injections were made onto a 30-m DB-5ms capillary column (0.25 mm inner diameter, 0.25 µm film thickness) with an HP 18596A Autosampler (Hewlett-Packard). Quantification of compounds was conducted by comparing the response of the analyte to the response of the deuterated coinjection standard that most closely matched its retention time. To correct for the varying response factors encountered for compounds with different molecular structures, we calibrated the instrument using authentic standards purchased from Aldrich Chemical (Milwaukee, WI) and the National Institute of Standards and Technology (SRM 2260; Gaithersburg, MD)

Meteorologic data. The morning mixing height data examined during the course of the study, processed from the surface and upper air meteorologic data at Santa Teresa, New Mexico, was obtained from the National Climatic Data Center, Asheville, North Carolina. Santa Teresa, located in the northwest corner of the Paso del Norte airshed, provides the only available surface meteorologic data for the region. Mixing height measures the height above the ground level in which air freely mixes. The correlation between mixing height and *in vitro* bioassays was made.

Statistical analysis. We used Pearson's correlation coefficients to examine the relationship between the gravimetric analysis of PM_{10} and the response variables. Simple regression analysis was used to identify a relationship between morning mixing height and luciferase activity. We did not perform statistical analyses on bioassay data because samples for each date and location came from a single filter. We used SAS software and Statview (both from SAS Institute, Inc., Cary, NC) to perform the analyses.

Results

We determined the gravimetric results by weighing the filters before and after PM_{10} collection for filters obtained from the three sites on the 15 collection dates (data not shown). No exceedances of the U.S. EPA 24-hr standard of 150 µg/m^3 of air occurred at the Tillman or Northeast sites; however, four exceedances were observed at the Advance site. In general, PM_{10} (by weight) collected at the Advance site was 6–8-fold greater than that collected at Northeast and 2–3-fold greater than that collected at Tillman.

Results of the range-finding experiments are shown in Figure 1. The DCM extracts from Northeast and Advance that were equivalent to 0.03–0.17 m^3 air caused an increase in EROD activity. EROD activity in Tillman extracts declined over most of the concentration range. On the basis of this study, we exposed H4IIE cells to extract equivalent to 0.146 m^3 air for the remainder of the experiments. In contrast to the decline in activity observed with EROD at higher amounts of extract, no such inhibition was seen with luciferase activity.

Cytotoxic effects were noted only when cells were exposed to extracts equivalent to 0.47 m^3 air from the Tillman and Advance sites, as indicated by the reduced metabolism of tetrazolium dye in the XTT assay. We also noted increased vacuolization of H4IIE cells exposed to more than 5.0 m^3 air collected from Tillman and Advance (data not shown). In contrast, no inhibition or changes in cellular ultrastructure were observed with extracts from Northeast at any concentration. We observed no cytotoxicity at extract doses used in either the EROD and luciferase assays, which were 3- and 32-fold lower, respectively, than the lowest cytotoxic dose.

The effect of exposures of H4IIE cells to extracts prepared from filters collected from the three monitoring sites over the 3-month period on EROD activity is shown in Figure 2. With one exception (23 February), EROD activity in cells exposed to Advance extracts was highest among the three sites. EROD activity associated with the Northeast site was consistently the lowest of the three for any single collection date. Typically, results obtained from Tillman samples fell between the two other values. On average, EROD activity from Tillman extracts was about 1.5 times greater than Northeast, and Advance extracts were 1.5–2 times greater than those from Tillman. Only extracts from Advance approached the activity of 11 pmol/min/mg protein, seen with the positive control, 1 µM BNF. We have shown that this concentration of BNF produces a similar degree of AhR induction as 10 nM TCDD (Garrison et al. 1996). Analysis of activity from Advance samples on 2 of the 5 days in January had similar levels of activity as cells treated with 1 µM BNF (Figure 2B). EROD activity measured in Northeast POM obtained from the PM_{10} filters was the most consistent over time, whereas EROD activity of Advance POM was the most variable.

The effects of exposures of extracts to H1L1.1c2 cells containing the inducible luciferase reporter system are shown in Figure 3. A similar pattern of induction was observed in these experiments as was obtained in the EROD assay. Luciferase activity in samples from Tillman was 1.5 times greater than that in Northeast samples; activity seen with

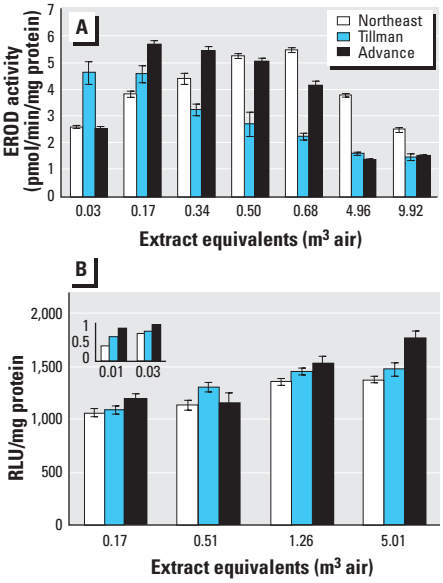

Figure 1. Effects of varying amounts of PM extracts from 7 December 1998 on bioassays. (*A*) EROD activity in H4IIE cells exposed to extracts from particles collected from 0.03–9.92 m^3 filtered air for 24 hr. (*B*) Luciferase activity in H1L1.1c2 cells containing an AhR-driven luciferase reporter system exposed to extracts containing between 0.01 and 5.01 m^3 filtered air for 4 hr. Bars represent mean ± SEM of four replicates.

Advance extracts was about 1.5 times that measured for Tillman. Again, the exception to this rule was noted with the 23 February sample, where Tillman was highest and the difference between Advance and Northeast was only 50%. The fluctuations in activity at Advance seen with the EROD assay were not as great with the luciferase assay.

There was a significant relationship between PM_{10} mass (μg/m^3 in 24 hr) and both EROD and luciferase activities (Figure 4). When data from all three sites were pooled, we observed a positive correlation between PM_{10} mass and the two major end points ($p < 0.0001$). However, when this relationship was analyzed by site, the relationship was not as strong, primarily because of clustering of data at the lower or higher end of the PM_{10} mass range. We found a statistically significant relationship between luciferase activity and filter weight at the Advance and Tillman, but not at Northeast (data not shown).

To gain some insight into the role winter inversion layers might have on PM_{10} and associated POM, we examined the relationship between morning mixing height and luciferase activity (Figure 5). During the winter, morning mixing height was very low, averaging < 100 m most of the time. As a comparison, morning mixing heights of 250–350 m are common during the summer months. Different relationships between mixing height and activity were found at the three sites. The strongest relationship was observed at Tillman ($p < 0.006$), a strong association was seen at

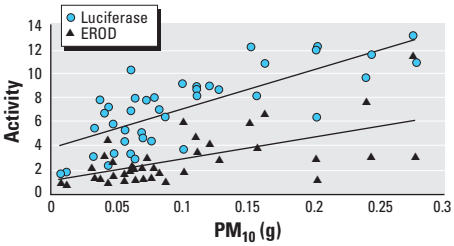

Figure 4. Relationships between PM_{10} gravimetric weight and bioassay activities. EROD units, pmol/min/mg protein; $r = 0.60$**. Luciferase units, RLU × 10^{-2}/mg protein; $r = 0.76$**. Each data point represents the mean of four replicates.
**Significant difference from zero at $p < 0.001$ level.

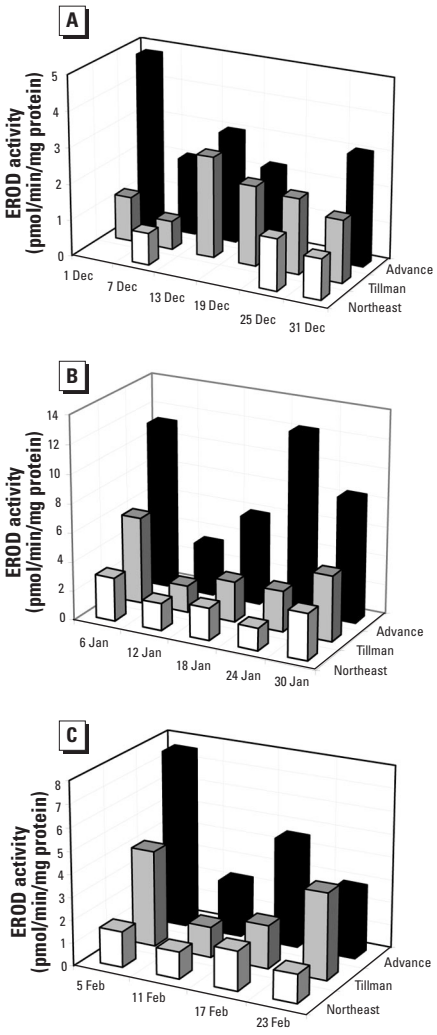

Figure 2. Effects of PM_{10} extracts on EROD activity in H4IIE cells exposed to extracts equivalent to 0.145 m^3 air from the three PM_{10} monitoring sites. (*A*) EROD activity associated with December 1998 filter collections. (*B*) Data from January 1999 filter collections. (*C*) Data from February 1999 filter collections. Negative control activity was 1.14 pmol/min/mg protein; positive control activity with 1 μM BNF was 11 pmol/min/mg protein. Data points represent the mean ± SEM of four replicates.

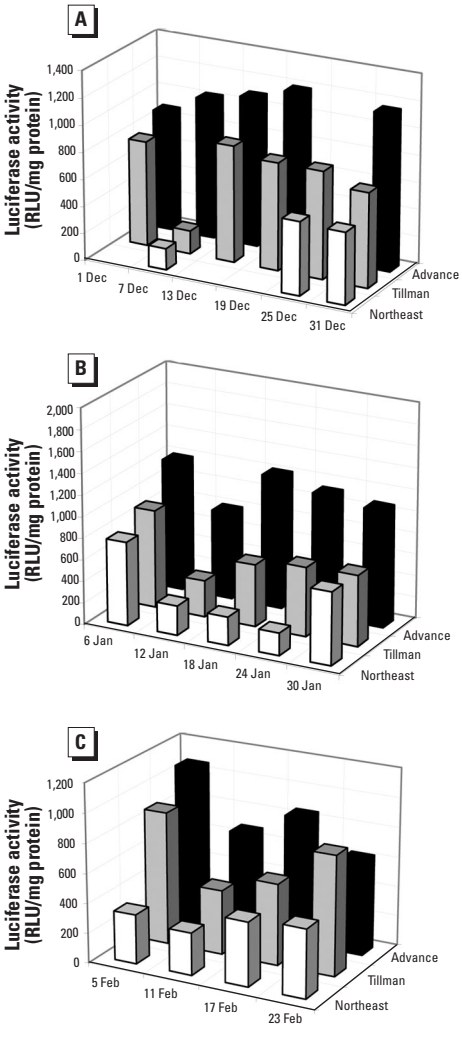

Figure 3. Effects of PM_{10} extracts on luciferase activity in H1L1.1c2 cells exposed to 0.0145 m^3 air from the three PM_{10} monitoring sites. (*A*) Luciferase activity associated with December 1998 filter collections. (*B*) Data from January 1999 filter collections. (*C*) Data from February 1999 filter collections. Negative control activity was 18 RLU/mg protein; positive control with 1 μM BNF was 1,810 RLU/mg protein. Data points represent the mean ± SEM of four replicates.

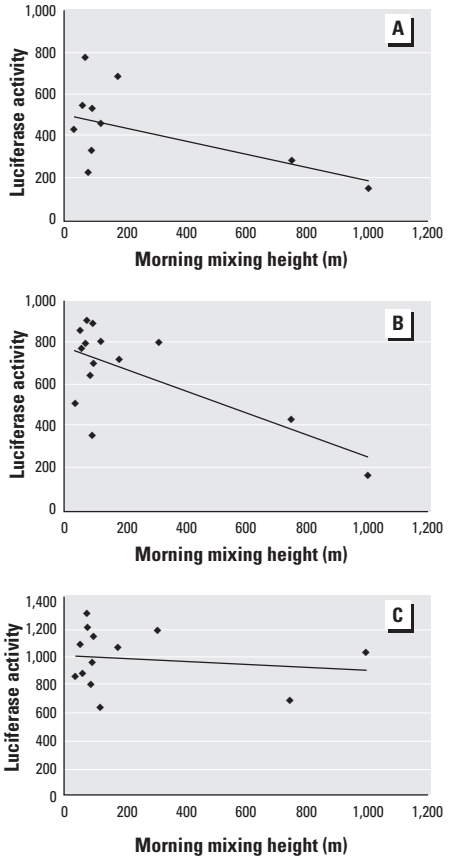

Figure 5. Relationship between morning mixing height and luciferase activity. (*A*) Northeast site ($y = -0.315x + 505$; $R^2 = 0.29$). (*B*) Tillman site ($y = -0.51x + 778$; $R^2 = 0.475$). (*C*) Advance site ($y = -0.12x + 1{,}020$; $R^2 = 0.029$). Data points represent the mean of four replicates. Mixing height data were obtained from the National Climatic Data Center in Asheville, North Carolina; upper air data were retrieved from Santa Teresa, New Mexico [National Weather Service (NWS) Station number 03020], and the surface meterorlogic data were retrieved from El Paso, Texas (NWS Station number 72270).

Northeast ($p < 0.08$), and no relationship was noted at Advance.

Finally, we measured the concentrations of the target PAH analytes for nine collection dates in POM collected from the Advance filters (Table 1). Sample dates are ordered according to the PM_{10} collection on the respective date. In general, the total measured PAH concentration increased with PM_{10} mass. PAHs detected in the highest concentrations were benzo[*a*]pyrene (BaP), chrysene/triphenylene, benzo[*b*+*j*+*k*]fluoranthene, and benzo[*g*,*h*,*i*]perylene. One sulfur-containing and three oxygenated PAH compounds were also analyzed. The molecular weight of the compounds investigated ranged from 234 kDa for retene (1-methyl-7-isopropyl-phenanthrene) to 300 kDa for coronene. Total PAH concentration was also reported for each date. We found a positive correlation ($R^2 = 0.53$, $p < 0.03$) between PAH concentration and luciferase activity (data not shown). On average, the concentration of PAHs was 12.7 ng/m^3. As a reference, data from PM_{10} extracts collected in three other cities in the Americas are also provided in Table 1.

Discussion

One of the major goals of this study was to characterize the nature and biologic activity of POM in the Paso del Norte airshed. Activation of the AhR is the key determinant in mediating the toxic and biochemical effects of dioxins and related PAHs (i.e., their carcinogenicity and endocrine-disrupting effects) (Machala et al. 2001). The value of measuring AhR activation is that it is integrative, taking into account atmospheric and biologic transformation of parent compounds, antagonistic or synergistic effects within the cell, and the ability to sum the biologic effects of xenobiotics, which is difficult to accomplish by chemical analysis.

As expected, activities of both EROD and luciferase were greatest in samples from the Advance site, the PM_{10} station adjacent to a brick-making district in Juarez (Figures 2 and 3). In January, the month in which the highest activity was recorded, luciferase activity was 1.7 times that measured in the Tillman samples and 2.5 times greater than measured for the Northeast. EROD activity at Northeast was consistently low, similar to control activity of nonexposed H4IIE cells (0.85 pmol/min/mg protein). However, luciferase was higher than control activity (58 RLU/mg protein), suggesting that small amounts of AhR ligands were present in Northeast extracts. EROD and luciferase activity associated with Tillman filters generally fell between the response observed at the other two sites. The average luciferase activity associated with DCM extracts was 432 RLU/mg protein from Northeast, 663 RLU/mg protein from Tillman, and 994 RLU/mg protein from Advance. This level of induction was caused by exposure to extracts equivalent to 0.145 m^3 of air and represents 24%, 37%, and 55%, respectively, of the activity seen with 1 µM BNF. These comparisons suggest significant amounts of AhR ligands are present in POM, especially at the Advance site.

Both luciferase and EROD activities mirrored total PM_{10} mass, as reflected by the significant positive correlation between these variables (Figure 4). This relationship suggests that AhR ligands contribute a constant percentage of the total POM associated with PM_{10}. In a location such as the Paso del Norte airshed, we speculated that the contribution of silica-based particles in PM might vary because of the desert terrain and high percentage of unpaved roads. However, our findings suggest that this is probably not the case, at least not in the winter months. It would appear that as total PM_{10} increases, there is a concomitant increase in PAHs and other AhR ligands.

To gain a better understanding of the potential for deleterious effects of the POM that induced AhR-mediated activity measured in this study, we identified work of other investigators who analyzed PAHs in PM extracts from a number of cities. Massolo et al. (2002) assessed the chemical composition, mutagenicity, and toxicity of particulates from La Plata, Argentina, and Leipzig, Germany. Extracts from industrial areas in La Plata contained approximately 2.0 ng BaP/m^3 of air collected via a high-volume sampler (Massolo et al. 2002). BaP in our samples collected from Advance ranged from 0.19 to 4.48 ng BaP/m^3, with a geometric mean of 1.3 ng BaP/m^3. Extracts from the winter La Plata samples were used for mutagenicity testing using the TA 98 strain of *Salmonella typhimurium* with an S9 microsomal fraction (TA 98 + S9); the results showed an average of 2.5 revertants/m^3 compared with 0.16 revertants/m^3 at the reference site. Massolo et al. 2002 found a significant correlation between the levels of carcinogenic PAHs and BaP and mutagenicity. They also found that the highest mutagenicity was associated with particles ≤ 1.5 µm in diameter, those that have the highest likelihood of reaching the alveoli and deep respiratory tract. In a study in La Spezia, Italy, Barale et al. (1991) found BaP concentrations of 1.6 ng/m^3 at one of their study sites, a concentration comparable with

Table 1. Comparison of PAH values in the Advance site PM_{10} air samples compared with literature values of other cities.

				Advance site PAHs (ng/m^3)									
Date	Santiago, Chile 1996[a]	Denver, Colorado 1997[b]	Toronto, Canada 1998[c]	1 Dec 1998	7 Dec 1998	19 Dec 1998	31 Dec 1998	6 Jan 1999	18 Jan 1999	24 Jan 1999	11 Feb 1999	17 Feb 1999	GM
PAHs													
Retene	–	0–0.8	–	0.4	0.2	1.0	0.9	1.2	ND	0.2	0.1	0.1	0.3
Benz[*a*]anthracene	4.8	0.04–2.9	0.07	0.45	0.4	2.4	1.9	3.1	0.6	1.0	0.1	0.2	0.7
Chrysene/triphenylene	1.3	0.1–1.8	0.15	0.6	0.6	2.8	2.4	3.6	0.9	1.3	0.2	0.4	1.0
Benzo[*b*+*j*+*k*]fluoranthene	8.6	0.4–2.7	0.40	3.0	1.5	7.9	5.3	7.4	3.0	4.9	0.5	2.2	3.0
Benzo[*e*]pyrene	–	0.1–1.6	0.18	1.2	0.5	2.7	2.4	3.3	1.3	1.7	0.2	0.8	1.2
Benzo[*a*]pyrene	4.9	0.1–1.5	0.06	1.0	0.5	3.9	2.9	4.5	1.8	2.3	0.2	0.5	1.3
Perylene	–	–	0.02	0.2	0.1	0.7	0.6	0.8	0.4	0.5	0.04	0.1	0.3
Dibenz[*a*,*h*]anthracene	4.5	–	0.03	0.1	0.05	0.5	0.2	0.4	0.1	0.3	ND	0.1	0.2
Indeno[1,2,3-*cd*]pyrene	6.7	0.03–1.2	0.25	1.3	0.4	2.4	0.2	3.1	1.5	1.9	0.2	0.7	0.9
Benzo[*ghi*]perylene	7.6	0.1–2.8	0.15	2.6	0.9	4.4	3.2	4.9	2.2	3.0	0.4	1.4	2.0
Coronene		0.1–1.6		1.2	0.5	ND	1.4	1.9	1.0	1.2	0.2	0.7	0.8
Sulfur-containing PAHs													
Benzonaphthothiopene	–	–	–	0.2	0.04	ND	0.3	0.3	ND	0.4	ND	0.5	0.3
Oxygenated PAHs													
Benzanthrone	–	–	–	0.3	0.2	0.9	0.8	1.3	0.2	0.5	0.1	0.2	0.3
Benz[*a*]anthracene-7,12-dione	–	–	–	0.1	0.1	ND	0.2	0.4	0.1	0.3	0.3	0.1	0.05
1,4-Chrysequinone	–	–	–	0.1	ND	0.1	0.1	0.2	ND	0.1	ND	ND	0.1
Sum of PAHs	–	–	–	12.8	5.9	29.9	23	36.1	13.3	19.6	2.3	7.5	12.7

Abbreviations: –, not reported; GM, geometric mean; ND, not detectable. Data represent analysis of a single aliquot of dichloromethane extract analyzed by GC/mass spectography from filters collected between December 1998 and February 1999. The values for all compounds are reported as nanograms per cubic meter. For comparative purposes, data from three other metropolitan areas are shown.
[a]Gil et al. 2000. [b]Chow et al. 1998. [c]Clemons et al. 1998.

our measurements at Advance. Although mutagenicity of samples with this level of BaP was not identified in their report, Barale et al. (1991) found that for approximately 10 ng/m^3 total PAHs (15 were measured), the number of revertants averaged 2.5/m^3 (TA 98 + S9). Gil et al. (2000) found a much higher level of mutagenicity in their study in Santiago, Chile. Using organic extracts from PM_{10} filters, they measured between 200 and 600 revertants/m^3 (TA 98 + S9) in samples with a concentration of about 5.0 ng BaP/m^3. These studies provide some frame of reference regarding the potential mutagenic effects that might be associated with the AhR-linked activities and PAH concentrations that were measured at the Advance site in this study.

PAHs identified in this study include some that are listed by the U.S. EPA as hazardous air pollutants because they have the potential to cause adverse health effects, including cancer, cardiopulmonary disease, and developmental and reproductive toxicity. The results of our studies suggest that neighborhoods located near industrial sites may be at increased risk for these environmentally linked conditions. One method of estimating the public health risk associated with exposure to PAHs is to calculate the risk of cancer. To do this, we used the U.S. EPA (1987) oral slope factor of 7.3×10^0 mg/kg/day and adjusted this value for the weight of a 70 kg male and an inhalation rate of 20 m^3/day in the following calculation: $(7.3 \times 70)/20 = 25.55$ mg/m^3/day or 2.56×10^{-5} ng/m^3/day. The concentrations of the seven major PAHs measured at the Advance site were converted using toxic equivalency factors based on U.S. EPA guidance to obtain BaP equivalents (U.S. EPA 1993). This value averaged 1.97 ng BaP equivalents/m^3 over the sampling period. Multiplying the BaP equivalents by the slope factor yielded the value of 5.03×10^{-5}, or 5 cancers per 100,000 people. This value represents an estimate of the number of cancers associated with a lifetime exposure to PAHs at the average concentration measured during the winter at Advance. Some of the limitations of this estimate include the fact that data for the slope factor were derived from oral exposures and that personal exposure is not necessarily at the same concentrations as those collected by the PM_{10} samplers. For comparative purposes, we used the World Health Organization (WHO) Air Quality Guidelines for Europe's unit risk factor of 9×10^{-5} per nanogram BAP per cubic meter (Borstrom et al. 2002) to make a second risk calculation from ambient PAHs; an estimate of 1.2 cancer cases per 10,000 was obtained (Borstrom et al. 2002). Although the factors used in the U.S. EPA and WHO calculations were based on different types of data, the results suggest comparable risks—between 5 and 12 cases per 100,000 people.

Limitations of this analysis include the fact that risk estimates are based on PAH concentrations measured in winter, when the combination of inversion layers, higher emissions from combustion sources, and reduced atmospheric degradation all lead to higher concentrations than would likely be found in other months of the year. Nonetheless, these estimates suggest a considerable risk associated with exposure to PAHs, especially in light of the fact that the U.S. EPA considers a cancer risk of between 1 in 100,000–1,000,000 as not significant; the State of California's Proposition 65 program (State of California 1986) uses 1 in 100,000 as the no-significant-risk level. This estimate can be compared with an estimate of cancer risk associated with urban life in industrialized nations (Hemminki and Pershagen 1994). These workers calculated a risk of one cancer case per million people per year among urban residents exposed to an average ambient air concentration of 0.7 ng BaP/m^3. Ambient concentrations of PAHs at other sites in the airshed as well as the concentration of other important toxic air pollutants, such as nitro-PAHs, PCBs, and dioxins, are not known and should be investigated in the future to obtain a more accurate estimate of risk.

In addition to cancer risk, developmental and reproductive toxicity are associated with PAH exposure. Three-day-old chick embryos exposed to crude extracts of urban air from Teplice, Czech Republic, containing carcinogenic PAH mixtures at a concentration of 49.5 ng/m^3, developed defects in the heart and abdominal wall (Binkova et al. 1999). Carcinogenic PAHs such as BaP were present at levels of 7.42 ng/m^3 during the winter in Teplice. Furthermore, carcinogenic PAHs at concentrations > 15 ng/m^3 have been known to attenuate fetal growth in humans (Dejmek et al. 1999). At Advance, the highest concentration of BaP was about one-half of this value, 4.88 ng/m^3, during the winter. The concentration of total PAHs was on average 12.8 ng/m^3, similar to that reported by Dejmek et al. (1999). In this study, white blood cell DNA adduct levels were higher in infants (7.9 ± 0.93 per 10^8 nucleotides) than in their mothers (5.9 ± 0.77 per 10^8 nucleotides). These findings suggest that infants might be at greater risk of DNA damage because of their reduced ability to detoxify these types of contaminants (Perera et al. 1999). The presence of these contaminants in the Paso del Norte airshed would suggest there is a risk for developmental toxicity that needs further investigation.

The chemical analysis of PM_{10} extracts collected from Advance showed that the concentration of most PAHs were higher than the average ambient concentration reported for Toronto (Clemons et al. 1998) and in some cases for Denver, Colorado (Chow et al. 1998). However, PAH concentrations at Advance were consistently lower than those found in Santiago (Gil et al. 2000). The upper limit of the concentrations in Toronto and Denver are comparable with the highest concentrations at the Advance site for benz[*a*]anthracene, retene, and coronene. For many of the other compounds, however, the higher concentrations at Advance are approximately two to three times higher than the three cities identified above. For example, the concentrations of benzo[*g+j+k*]fluoranthene and benzo[*g,h,i*]perylene on certain dates was approximately double the highest value reported in Denver. Consistent with other assessments of ambient air quality, the PAHs quantified here probably account for a small percentage of POM (Seinfeld and Pandis 1998).

Some of the PAHs identified are recognized markers of anthropogenic activities. Retene is a biomarker of wood smoke combustion and was observed in all samples in varying concentrations (Ramdahl 1983). Observed but not quantified in the samples were methoxylated phenols, also derived from wood combustion (Hawthorne et al. 1992). Wood and sawdust are a major component of fuel used in brick-making, and wood is used for heating and cooking in most homes in the vicinity of the Advance site. A number of other compounds were found that typically indicate the presence of motor vehicle emissions. Relatively large amounts of hopane/sterane compounds, products of burning oil by smoking vehicles and diesel trucks, were also observed (Seinfeld and Pandis 1998). The presence of this class of compounds could also be associated with the use of fuel oil in brick-making.

The interaction between emission sources and climatic conditions needs to be considered when evaluating the potential health effects of urban air pollutants (Moller et al. 1994). In particular, an understanding of the influence of meteorologic conditions could assist in estimating possible impact of inversion layers on the distribution and concentration of POM within the airshed. The most dominant weather characteristic in the winter in the Paso del Norte airshed is the presence of inversion layers during winter months, which regularly reduce mixing height to < 100 m. In the present study, different relationships between morning mixing height and luciferase activity were seen at the three sites. At Advance, no relationship was observed, suggesting that localized emissions, most likely associated with brick-making activity, were the main influence on the content of the POM. Regardless of the presence of an inversion layer, luciferase activity was consistently high. This contrasts with Tillman, where a positive correlation existed between the morning mixing height and luciferase activity. The Tillman site is located in downtown El Paso near the center of the

basin that forms the Paso del Norte airshed. It is within blocks of the Sun Metro main bus depot and a bridge, a major crossing between El Paso and Ciudád Juarez that has heavy truck and automobile traffic. Because of its location, one would anticipate that the presence of an inversion layer would significantly affect POM at Tillman.

In contrast to Tillman, the relationship between POM and the presence of an inversion layer was weaker at the Northeast site ($p < 0.08$), located near the edge of El Paso; normally PM_{10} is lowest in this area of the city. However, when inversion layers occur, it is apparent that PM_{10} generated in other areas disperses throughout the airshed and changes the character of POM at this site. What is not clear is the degree to which either *a*) particles generated by traffic and/or brick-making activity many miles away in Juarez influence the conditions at sites in El Paso or *b*) the degree to which mobile-source combustion by-products generated in El Paso affect POM in Ciudád Juarez during an inversion layer. The shallow winter inversion layer coupled with complex terrain topology, potential nighttime drainage flow, native vegetation detritus, vehicle-enhanced road dust, and increased emissions of biomass combustion and waste burning in the wintertime in Paso del Norte complicate the relationship between PM_{10} and AhR ligands (Li 1999; Li et al. 2001). Combining data generated by the present study with topographic and air dispersion models in a geographic information system (GIS) would be needed to answer this question. One example of this approach was used by researchers in Stockholm, Sweden (Bellander et al. 2001), who used GIS to assess historic exposure to nitrogen dioxide and sulfur dioxide in various neighborhoods throughout the city. Geographically coding the PM_{10} sampler sites throughout the airshed and including AhR-mediated activity in a GIS analysis would provide useful information regarding potential exposure of people throughout the airshed to harmful contaminants.

Conclusion

This work represents a first attempt to identify toxic chemicals associated with PM_{10} in the Paso del Norte airshed. Of the two bioassays used to detect AhR ligands, the luciferase reporter assay was the more sensitive. Our approach of using *in vitro* assays such as these to identify potential sources of POM provides rapid results and conclusions that are complementary and easier to achieve than the traditional, labor-intensive, source apportionment and principal component analysis. In addition, these cell bioassays can be used for screening large numbers of sample extracts to identify those for subsequent expensive instrumental analysis.

The results from our study suggest that the levels of AhR ligands in some portions of the Paso del Norte airshed could be linked to adverse health effects, including cancer, respiratory, allergic, and developmental effects. Our studies demonstrate the toxicologically active PAHs are present at different sites in the Paso del Norte airshed. Environmental health monitoring and remediation should focus on known or suspected point and non-point sources of these and other environmental pollutants.

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