Toxic Evaluation of Organic Extracts from Airborne Particulate Matter in Puerto Rico

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In recent years, several hypotheses have emerged to explain the toxicologic activity of particulate matter. Organic compounds, ultrafine particles, biologic components, and transition metals are some of the constituents that reportedly exert some type of adverse effect on human health. A considerable fraction of the urban particulate matter consists of carbon compounds, which originate mostly from anthropogenic sources. The toxicity of organic fractions from particulate matter have been mainly evaluated by considering their mutagenic activity. This research expands on the toxicologic profile of organic compounds adsorbed to particulate matter, specifically in Puerto Rico, by using the cytotoxic neutral red bioassay (NRB). The NRB uses normal human epidermal keratinocytes or other types of cells to measure the effect on cell viability when exposed to organic compounds associated to the particles in the air. We validated the NRB for particulate matter by using a standard reference material (SRM 1649). We used the NRB to determine toxicologic differences of extracts between an urban industrialized site with anthropogenic activity versus a coastal region with less human activity. The cytotoxicity associated with organic compounds in particulate matter collected at the urban industrialized site was detected in both the particulate matter ≤ 10 µm in aerodynamic diameter (PM_{10}) and particulate matter ≤ 100 µm in aerodynamic diameter (PM_{100}). Greater toxic effects were observed in PM_{10} extracts than in PM_{100} extracts, but PM_{10} toxic effects were not significantly different from those in PM_{100}. The extracts from the industrialized site were more cytotoxic than the extracts from coastal reference site, although in the summer, extracts from both sites were significantly cytotoxic to normal human epidermal keratinocytes. In addition, the nonpolar extracts of both PM_{10} and PM_{100} exerted the greatest cytotoxicity, followed by the polar, and, finally, the moderately polar extract. This study demonstrates that extracts from the Guaynabo industrialized site were more toxic than similar extracts obtained from a reference coastal site in Fajardo, Puerto Rico. *Key words*: air pollution, bioassay, cytotoxicity, human cells, organic extracts, particulate matter, PM_{10}, PM_{100}, SRM 1649, toxicity.
Environ Health Perspect 108:635–640 (2000). [Online 1 June 2000]
http://ehpnet1.niehs.nih.gov/docs/2000/108p635-640reyes/abstract.html

Air pollution in Latin American and Caribbean countries has been a major environmental health issue for people living in metropolitan areas. These health issues arise as a consequence of urban development, population growth, and industrialization. Epidemiologic studies suggesting airborne particulate matter as one of the principal causes of respiratory illnesses (*1,2*), cardiovascular disease mortality (*3,4*), acute bronchitis (*5,6*), and asthma attacks (*7–9*) have been controversial (*10*). The differences center on the meta-analysis performed on data from U.S. cities, London, and Athens, Greece. However, other meta-analytical studies recently showed that a relationship between air pollutants and health does exist after adjustment for numerous variables (*11–13*).

Several hypotheses from these epidemiologic data attribute health effects to active toxicologic components of particulate matter. These hypotheses have focused on identifying components of particulate matter responsible for the observed health problems. The factors suggested to cause these health effects are the associated organic components (*14–17*); ultrafine particle constituents (*18*); the presence of biologic material (*19*); and the transition metal content of particulate matter (*20*).

The organic fraction of urban airborne particulate matter has been evaluated with regard to the mutagenic and genotoxic activity of the compounds found in the various extracts. Results from this research indicate that semipolar and polar fractions exhibit the greatest toxicologic activity using the Ames test (*15–17*), sister chromatid exchange assay (*17*), and a human cell mutagenic assay (*14*). Organic compounds such as polyaromatic hydrocarbons (PAHs), substituted aromatic hydrocarbons, and heterocyclic aromatic compounds have been targeted for these assays. A more recent study showed evidence that biologically effective amounts of PAH compounds associated to particulate matter were transferred to the intracellular environment and elicited the activation of various genes (*21*). Previous studies have demonstrated with great detail the activation of the aryl hydrocarbon receptor by tetrachlorodibenzo-*p*-dioxin (*22*). The activation of specific genes by organic pollutants is an area of great interest and future development.

Ultrafine particles < 0.10 µm in diameter could cause an acute inflammatory reaction as measured by lung lavage parameters in rats (*18*). Researchers have demonstrated an increase in pulmonary toxicity with exposure to ultrafine particles and a correlation with the surface area of the retained particles rather than their mass, volume, or numbers.

Another possible source of toxicity by particulate matter is biologic components such as aeroallergens, which have been associated with asthma-related mortality (*19*). An epidemiologic study showed that during 1985–1989 in Chicago, the asthma-related deaths were associated with high mold spore counts. The death rate was 2.16 times higher than the death rates related to asthma at levels < 1,000 spores/m^3.

Finally, the metal portion of particulate matter, specifically the elements iron, nickel, and vanadium, causes pulmonary toxicity (*20*). Lung injury interactions were observed among these metals when tested as mixtures. The toxicity of metals can also be potentiated and enhanced by other factors such as organic material that can induce inflammation in the lungs of rodents (*23*). All of the aforementioned studies lead to the unified hypothesis that the toxicity of airborne particulate matter is multifactorial and is influenced by the nature and origin of the particles.

Epidemiologic data gathered from an urban area in Puerto Rico (Guaynabo) revealed that approximately 30% of children 13–14 years of age and > 40% of children 6–7 years of age suffer from asthma (*24*).

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We thank R. Delgado for technical assistance, the Puerto Rico Environmental Quality Board (particularly A. Morales) for providing filter samples, and the Conservation Trust of Puerto Rico for allowing the location of the samplers in a protected area.

This work was supported by EPA-EPSCoR (R821843-01-0) and RCMI (RR-03051). D.R.R. acknowledges the pre-doctoral fellowship provided by the NASA-EPSCoR Program (NGT40053).

Received 11 August 1999; accepted 7 March 2000.

Furthermore, the U.S. Centers for Disease Control and the Puerto Rico Health Department reported that almost 50% of the children from this area have learning disabilities (*25*). In addition to this finding, they report that 41% suffer from one or several medical conditions (as compared to 23% in the rest of the island), and that there are more types and cases of cancer in this community than in other regions of Puerto Rico. The incidence of respiratory diseases reported in this area and the relatively high concentration of airborne particulate matter are higher than other regions of Puerto Rico, suggesting a direct link between these health conditions and airborne particulate matter at Guaynabo. The problem is exacerbated even further by susceptibility factors; Puerto Rican children are more affected by asthma than are other Latino children (*26*). This difference is attributed to a combination of unique genetic and sociocultural factors, such as smaller airway size, more severe inflammatory reactions, and lower birth weight than that of Cuban-American or Mexican-American children.

Most of the toxicity tests performed on urban airborne particulate matter have been based mainly on traditional mutagenic tests using the *Salmonella*/microsome bioassay (*27–32*). Although this is a useful and informative test, it only measures the mutagenic potential of compounds tested and does not consider direct toxicologic effects to human cells or systems. We used the neutral red uptake bioassay (NRUB) with primary cell cultures to determine cellular toxicity (lysosomal activity) caused by the exposure of organic components of airborne particulate matter from an urban polluted area and a coastal reference environment (Fajardo). Primary cultures of cells obtained from normal human tissues maintain many of the complex functions and biochemical systems that are active *in vivo*. The *in vitro* NRUB has been used to determine the cytotoxic effect on a myriad of chemical moieties such as organic (*33–38*) and inorganic chemicals (*39*), metals (*36,39–42*), pesticides (*34*), polycyclic aromatic hydrocarbons (*37*), food additives (*43*), chemotherapeutic agents (*44,45*), drug activity (*45,46*), and structure–activity correlation of toxins (*47*). Results from this assay have been compared to those of other known toxicologic assays, and the assay has been used with many cell lines including human hepatoma and melanoma tumor cells (*45,47*), normal human melanocytes (*45,47*), normal human epithermal keratinocytes (*35,43,45,47*), human fibroblast cells (*43,45,47*), human kidney cells, human endothelial cells, and human renal carcinoma cells (*47*), among others.

The results presented here encompass the toxicity of extracts obtained from two sites (Guaynabo and Fajardo) that contain low anthropogenic inputs of airborne particulate matter. This work is the first step to evaluate the toxicity of organic constituents from airborne particulate matter at two specific sites, and represents the first study of this nature in Puerto Rico. It approaches the characterization of toxicity of organic extract constituents based on differences in polarity using a cytotoxicity assay. The cytotoxicity of the extracts was seasonally dependent; summer components were the most toxic. Through this approach we conclude that extracts from the Guaynabo site are more toxic to human cells than those obtained from the Fajardo site.

Materials and Methods

Sampling procedure. Airborne particulate matter samples were collected at two sites, an urban industrialized area (Guaynabo), which is approximately 2 miles south of the entrance to the San Juan Harbor, and a reference site (Fajardo) on the east coast of Puerto Rico (Figure 1). Fajardo is on the coast and receives trade winds from the Atlantic Ocean; the only expected input is from boating activities, which occur mainly during the weekends. We simultaneously collected total suspended particulate matter [particulate matter ≤ 100 µm in aerodynamic diameter (PM_{100})] and inhalant particulate matter ≤ 10 µm in aerodynamic diameter (PM_{10})] at these sites during weekdays between May 1996 and September 1997. Sampling was performed for 48 hr on glass fiber filters using high-volume samplers and size-selective inlets; the samplers were placed 10 ft above the ground. All of the air filters were handled with latex gloves, immediately transported on dry ice, and stored in the laboratory at -20°C until processed. Sampling rates were on the average of 1.48 m^3/min for PM_{100} and 1.05 m^3/min for PM_{10}. We determined the amount of particulate matter gravimetrically by weighing the filters before and after sampling. Filters obtained from both stations and an urban dust standard reference material [SRM 1649; National Institute of Standards and Technology (NIST), Gaithersburg, MD] was extracted using a Soxhlet apparatus with solvents of different polarity (HPLC grade; Fisher Scientific, Fair Lawn, NJ). One-fourth of each filter was sequentially extracted for 24 hr with 175 mL hexane (nonpolar), dichloromethane (DCM) (moderately polar), and acetone (polar). Each extract was reduced to approximately 10 mL using a rotatory evaporator under vacuum and then dried completely under a gentle stream of nitrogen. The extracts obtained after this process were weighed and dissolved in 100 µL DMSO (ACS reagent; Sigma, St. Louis, MO). Several dilutions of the dissolved DMSO extracts were prepared with cell culture medium for NRUB cytotoxicity screening. The DMSO in the cell culture medium was always kept below 1% to avoid cytotoxicity of the carrier solvent. Blank samples prepared by extracting unused filters showed no NRUB toxicity.

NRUB. The selection of human primary epidermal keratinocytes for this study was based on two facts. First, these cells possess xenobiotic detoxification systems that would also contribute to the toxicity of metabolites transformed by biologic systems (*48*); hence, the use of these cells integrates the effects of both the parent xenobiotics and their metabolic products. Second, the amount of information that exists on a great number of compounds using keratinocytes is extensive and allows for comparisons with particulate matter extracts. We obtained human epidermal keratinocytes and all solutions used in the NRUB from Clonetics Corporation (San Diego, CA). Cells were treated as follows: keratinocyte growth medium (KGM) was supplemented with epidermal growth factor, insulin, hydrocortisone, antimicrobial agents, and bovine pituitary extract. Cells were subcultured in KGM at 37°C and 5% CO_2. We added fresh growth media on alternate days. At culture growth between 50 and 80% of confluence, cells were trypsinized, counted, and seeded onto 96-well tissue culture microtiter plates at approximately 3,000 cells/well. After 3 days, the medium was replaced with supplemented KGM media containing different concentrations of extracts. Cells were exposed to compounds in the extracts for periods of 36–48 hr. Each batch was treated with Triton X (a positive control), which is a known toxicant [concentration that kills 50% of the cell population (NR_{50}) between 3 and 32 µg/mL]. We also examined negative controls (untreated cells) on each plate. After exposure time, the extracts from

Figure 1. Sampling location sites. The urban site (Guaynabo) is immediately south of the San Juan Harbor, and the reference site (Fajardo, coastal) is on the northeast tip of the island.

particulate matter were decanted and a neutral red dye solution of 100 µg/mL was added to all wells, with the exception of the blanks. Cells were incubated with the dye solution for 3 hr at 37°C and 5% CO_2. Viable cells incorporate the dye, whereas damaged cells do not. After the incubation period, we removed the neutral red dye solution and fixed the cells. A solvent solution containing acetic acid and ethanol was added to the cells to allow the release of the neutral red dye. We determined the absorbance of the neutral red dye in each well spectrophotometrically at 540 nm, using a microplate reader. The amount of viable cells in each well was proportional to the absorbance readings. The absorbance of untreated cells was used to determine the percent of viability at each dose analyzed. The percent of viability of cells was obtained by dividing the average absorbance of wells at each extract dose by the average absorbance of the negative controls. The NR_{50} concentration value was obtained from a percent viability curve. Quantitation was determined as previously described (*47*). We used four replicate wells per dose of extract for determining the NR_{50} concentration.

Statistics. We used unpaired *t*-test or Mann-Whitney and analysis of variance tests to evaluate statistical differences between concentrations among sites. We evaluated differences between seasons and sampling sites. The statistical analyses were performed using the StatView computer software package (version 5.0; SAS Institute, Inc., Cary, NC). We established the probability value of $p < 0.05$ as the level for statistical significance.

Results and Discussion

Particle characterization per site. The monthly average concentration of PM_{10} (in micrograms per cubic meter) collected at Guaynabo and Fajardo during the years 1996–1997 is shown in Figure 2. By comparing the differences between the average monthly concentrations throughout the year for both sites, we determined that the amount of PM_{10} collected at Guaynabo is 34% higher than that obtained at Fajardo. The PM_{10} concentrations obtained for Guaynabo and Fajardo using our sampling procedures were not significantly different from those obtained by the Puerto Rico Environmental Quality Board (EQB) (*49*). Therefore, the amount of PM_{10} that was used in our assays (collected during 48 hr) was within the expected range of material normally reported by the local environmental agency.

Because PM_{100} regulations were no longer required by the state regulatory agency after 1993, we combined the available EQB Guaynabo data for 1993–1995 to generate an average PM_{100} profile with which to compare our results. All of the material obtained during our sampling period (fall, winter, spring, and summer) fluctuated between the average monthly concentration obtained from the 1993–1995 PM_{100}-generated profile at the Guaynabo site. Statistical analyses showed no significant differences among the PM_{100} concentration at Guaynabo during the years of 1993–1995 and our PM_{100} concentration at the same site (1996–1997).

A comparison of seasonal variations clearly indicates that the highest amount of airborne particulate matter (PM_{10} and PM_{100}) is evident during the summer season (Figure 2). The average concentration of particulate matter obtained during the summer was higher than that obtained during other seasons. This finding could be partially explained by the increases due to normal chemical and photochemical oxidation processes occurring during the warm season (*50*). Although our data identify the summer as containing the highest levels of particulate matter, the PM_{10} concentrations at both sites did not exceed the maximum daily permissible limit established by the EQB (50 µg/m^3) (*51*). However, the levels of PM_{100} for Guaynabo exceeded the maximum concentration of 150 µg/m^3 (secondary standard established to protect public welfare). The second highest concentration of particulate matter among the seasons was observed during the fall, followed by winter and spring. Thus, the trend observed in this study for levels of particulate matter for both PM_{10} and PM_{100} in Puerto Rico followed the order: summer > fall > winter = spring (Figure 3).

When these two sites in Puerto Rico are compared between themselves, we observe similar PM_{10} values between the urban and coastal site. Furthermore, PM_{100} concentrations from Fajardo were always lower than those from Guaynabo. Hence, the major profile of particles at the urban site is between 10 and 100 µm (Figure 3). We observed characteristic patterns, in terms of the PM_{10}/PM_{100} ratio, at both sites. Fajardo (coastal site) samples exhibited ratios in the range of 0.72–0.84, whereas samples obtained from Guaynabo exhibited lower ratios—between 0.35 and 0.44. These data indicate that most of the particulate matter sampled at the Fajardo site consists mainly of fine particles (PM_{10}), whereas that of Guaynabo is composed of larger particles. These results suggest that particulate matter obtained from each station originates from

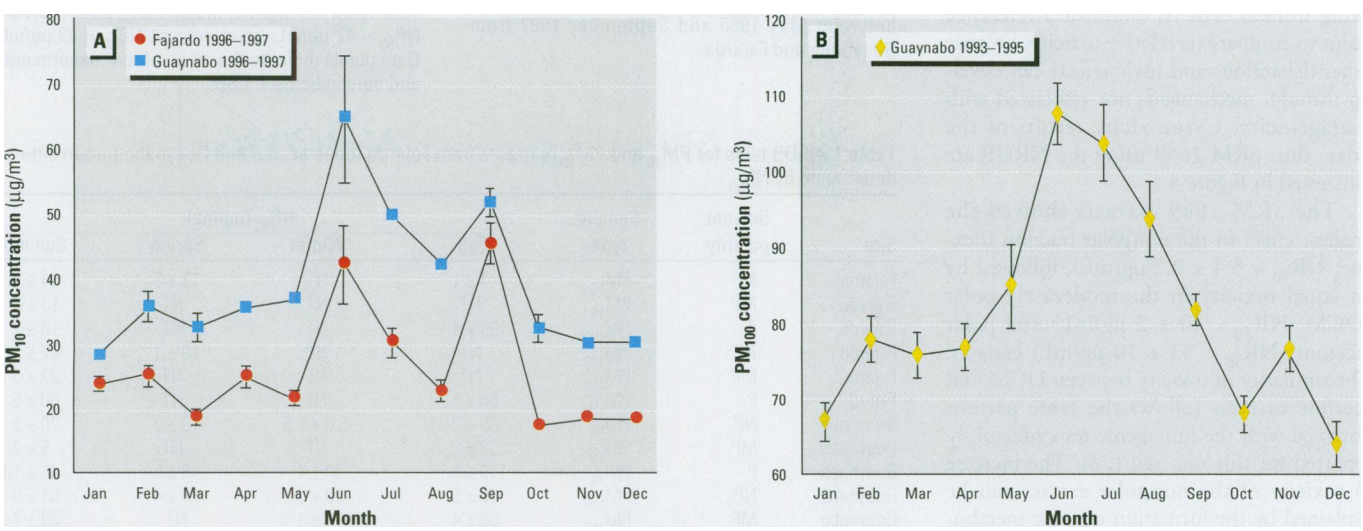

Figure 2. (*A*) Average PM_{10} concentration for Guaynabo and Fajardo for the years 1996–1997. Each point per month represents the average of at least 56 values for Guaynabo (n = 695) and 9 for Fajardo (n = 197) ± SE. When error bars not shown, SE < 1. (*B*) Average PM_{100} concentration per month for the Guaynabo. These data include information for three consecutive years (1993–1995). Each time point represents the average of at least 10 observations, with a total of 159 observations.

different sources. The particulate matter at the reference site (predominantly PM_{10}) is believed to contain organic compounds of marine origin, some anthropogenic compounds, and, occasionally, Saharan dust (*52,53*), whereas particulate matter collected at the industrial site is mostly of anthropogenic origin. Comparison of the PM_{10}/PM_{100} ratios from the industrialized site in Puerto Rico (0.35–0.44) tend to be slightly lower than other ratios obtained from other metropolitan sites (0.50–0.60) (*54*). The PM_{10} size distribution profile has not been performed for any area monitored in Puerto Rico. Preliminary gas chromatography/mass spectrometry results for the extracts at the Guaynabo site show that some of the compounds mostly found there are: bis(2-ethylhexyl) phthalate; malathion; dibutyl phthalate; 4-morpholinepropanamine; 6-undecylamine; 1-[3-methyl-4-(4-morpholinyl)-1-oxo-2,2-diphenylbutyl]-pyridine; and tridecane. Their concentrations range between 1 and 100 ng/m^3.

Toxicologic characterization. Urban dust standard reference material (SRM 1649) obtained from the NIST contains a series of organic compounds including PAHs and inorganic elements (heavy metals). The mutagenic activities (Ames test) of the moderately polar (DCM) and polar extracts (acetone) from this material are relatively similar (20–23 revertants/µg) (*28*). Furthermore, the mutagenic activity of the reference material was 3–4 times higher than that of airborne particles collected from a New Jersey site (*27*). These data provide evidence that moderately polar and polar fractions from urban dust (SRM 1649) contain mutagenic organic compounds. However, the cytotoxicity of these fractions has not been reported for human cells. Thus, it is important to establish the cytotoxicity of this material using human cells to establish a reference point to compare its relative toxicity, because other deleterious and toxic effects can develop through mechanisms not associated with mutagenicity. Cytotoxicity results of the urban dust SRM 1649 using the NRUB are illustrated in Figure 4.

The SRM 1649 extracts showed the greatest effect in the nonpolar fraction (hexane; NR_{50} = 5.4 ± 0.2 µg/mL), followed by an equal toxicity in the moderately polar (DCM; NR_{50} = 40 ± 2 µg/mL) and polar (acetone; NR_{50} = 43 ± 10 µg/mL) extracts. The similarity in toxicity between DCM and acetone extracts follows the same pattern observed with the mutagenic tests previously reported for this material (*28*). The increase in toxicity of the nonpolar extract can be explained by the formation of toxic metabolites of organic compounds inherent in this reference material. This is supported by the fact that human cells (primary keratinocytes) contain detoxification enzymes such as cytochrome P450s that are responsible for the metabolism of many foreign compounds (*55*). A previous study performed by Clonetics (*56*) using 52 petrochemical agents of different nature (lube oil additives, gasoline additives, polybutylene, solvent-refined paraffinic petroleum oil, and various metal-working fluids) suggested a direct relationship between the Draize score (*in vivo* eye irritation assay) and the NR_{50} using the NRUB. The Clonetics study showed that NR_{50} values > 150 µg/mL were nontoxic. Using this threshold, all three SRM 1649 extracts can be considered highly cytotoxic.

Table 1 shows a summary of the cytotoxic data for the PM_{10} and PM_{100} extracts obtained using the NRUB assay for both the Guaynabo and Fajardo sites throughout the various seasons. Forty-eight extracts were assayed during a 1-year period. Of all extracts evaluated, only 46% of the Fajardo coastal site samples were cytotoxic (NR_{50} < 150 µg/mL), whereas 21 of 24 or 88% of the samples from the Guaynabo station exhibited significant cytotoxicity (NR_{50} < 150 µg/mL). Generally, extracts from material collected in fall from the Guaynabo site were more cytotoxic than extracts collected at Fajardo during the same season. This is supported by the fact that many extracts collected from the Fajardo coastal site in the fall were nontoxic, with the exception of three fractions (Table 1). All PM_{10} and PM_{100} extracts from Guaynabo in the fall were cytotoxic to normal human epidermal keratinocytes. None of the PM_{10} or PM_{100} extracts from Fajardo exhibited cytotoxicity during winter. Conversely, all extracts of both PM_{10} and PM_{100} fractions from Guaynabo were very cytotoxic, with the exception of the moderately polar PM_{10} extract. This finding argues that the toxicity of particulate matter in Guaynabo during the winter comes from urban sources.

In general, extracts of the Fajardo coastal site samples showed that the nonpolar and moderately polar extract of the PM_{10} and the nonpolar and moderately polar extract of PM_{100} were significantly cytotoxic. For extracts collected from the industrialized Guaynabo site, the nonpolar and moderately polar extract of PM_{10} and the nonpolar and polar extracts of PM_{100} were significantly cytotoxic. In the summer, all extracts (PM_{10} and PM_{100}) from both

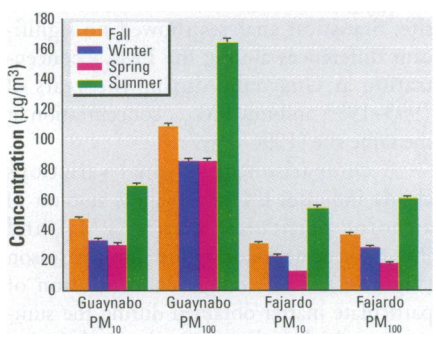

Figure 3. Concentration of PM_{100} and inhalable PM_{10} in micrograms per cubic meter collected between May 1996 and September 1997 from Guaynabo and Fajardo.

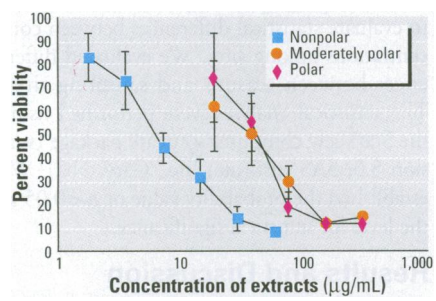

Figure 4. NRUB for the three extracts from the urban dust SRM 1649. The NR_{50} was determined at different extract concentrations: nonpolar solvent NR_{50} = 6 µg/mL; moderately polar solvent NR_{50} = 42 µg/mL; polar solvent NR_{50} = 43 µg/mL. Data points indicate the mean of four experiments and bars indicate ± 1 SD.

Table 1. NRUB tests for PM_{10} and PM_{100} extracts from Fajardo (coastal site) and Guaynabo (urban site) as determined by NR_{50}.

Site	Solvent polarity	Sample type	NR_{50} (µg/mL) Fall	Winter	Spring	Summer
Fajardo	NP	PM_{10}	4 ± 1	NT	13 ± 3	64 ± 2
Fajardo	MP	PM_{10}	NT	NT	NT	13 ± 2
Fajardo	P	PM_{10}	90 ± 4	NT	NT	18 ± 2
Fajardo	NP	PM_{100}	NT	NT	10 ± 1	42 ± 3
Fajardo	MP	PM_{100}	NT	NT	NT	27 ± 5
Fajardo	P	PM_{100}	66 ± 5	NT	NT	60 ± 5
Guaynabo	NP	PM_{10}	35 ± 2	5.8 ± 0.5	19 ± 2	20 ± 5
Guaynabo	MP	PM_{10}	32 ± 2	NT	NT	5 ± 2
Guaynabo	P	PM_{10}	119 ± 5	30 ± 1	46 ± 6	46 ± 10
Guaynabo	NP	PM_{100}	80 ± 5	58 ± 9	30 ± 4	51 ± 9
Guaynabo	MP	PM_{100}	59 ± 4	15 ± 1	NT	39 ± 7
Guaynabo	P	PM_{100}	49 ± 2	24 ± 1	23 ± 6	23 ± 3

Abbreviations: MP, moderately polar (DCM); NP, nonpolar (hexane); NT, nontoxic (determined by NR_{50} > 150 µg/mL); P, polar (acetone).

Fajardo and Guaynabo were cytotoxic (Table 1).

To further evaluate the relative toxicity of extract fractions by site and by season, we determined statistical differences between extract fractions per site and season. This analysis yielded an order of toxicity for each extract fraction (Table 2). Two major findings arise from this data. First, differences in the toxicity of extract fractions with respect to particle size were noticeable. An increase in toxicity was associated with increased polarity of extract constituents for PM_{100}. Conversely, extracts obtained from PM_{10} were more toxic as polarity decreased (hexane constituents are more toxic). This difference in toxicity could be explained by the type of chemical constituents partitioning into each solvent. For example, metals and polar organic compounds are expected to partition into more polar fractions, whereas other organics are associated to nonpolar fractions. The next major differences are those between sites. Extract fractions obtained from Guaynabo were toxic throughout the year; extracts from Fajardo were mainly toxic during the summer. Because the major toxicity of extracts was observed during the summer, we compared extract toxicity between sites during this season. Guaynabo had significantly higher toxicity in the nonpolar (hexane) and semipolar (DCM) fractions of PM_{10}, whereas Fajardo exhibited significantly higher toxicity in the polar extract. The most significant toxic effect of PM_{100} extracts between sites during the summer was observed in Guaynabo. This effect was observed with the polar extract fraction. Overall, comparable extract fractions between sites were more toxic at the Guaynabo site. The average NR_{50} for summer in the industrialized (urban) site was 31 ± 7 µg/mL; the overall average NR_{50} for Fajardo was 37 ± 11 µg/mL. This Guaynabo NR_{50} value is comparable to the NR_{50} reported for Triton X (3–32 µg/mL), a well-established toxic detergent used as a positive control with this assay. This NR_{50} toxicity value is also comparable to that obtained for extracts from our standard reference material (urban dust SRM 1649), which ranged from 5.4 to 43 µg/mL (Figure 4).

Table 2. Descending order of toxicity for PM_{10} and PM_{100} extracts from Fajardo (coastal site) and Guaynabo (urban site) based on NR_{50} values.

Site	Season	Particle size	Order of extract toxicity
Fajardo	Summer	PM_{10}	DCM
			Acetone
			Hexane
Guaynabo	Summer	PM_{10}	Acetone
			DCM
			Hexane
Guaynabo	Fall	PM_{10}	Acetone
			DCM
			Hexane
Guaynabo	Winter	PM_{10}	DCM
			Acetone
			Hexane
Guaynabo	Spring	PM_{10}	Acetone
Fajardo	Summer	PM_{100}	DCM
			Hexane
			Acetone
Fajardo	Fall	PM_{100}	Hexane
Guaynabo	Summer	PM_{100}	Acetone
			DCM
			Hexane
Guaynabo	Fall	PM_{100}	Acetone
			DCM
			Hexane
Guaynabo	Winter	PM_{100}	DCM
			Acetone
			Hexane
Guaynabo	Spring	PM_{100}	Acetone

In summary, although the NRUB is an *in vitro* assay that uses primary human cells, only considers one end point, and was not evaluated simultaneously with other toxicologic assays, it provided significant data to establish marked differences in seasonal samples and urban versus rural airborne particulate extracts. The cytotoxicity of organic compounds extracted from particulate matter is higher in the nonpolar extract component. This was shown for both SRM 1649 and the particulate matter extract from the industrialized site, particularly in the PM_{10}. Finally, we determined that the cytotoxicity was seasonal dependent; summer was the most toxic season at both sites studied. This work supports the hypothesis put forth by others that organic constituents adsorbed to particulate matter (particularly those of lower particle size) can contribute to the toxicity of airborne particulate matter. This work is the first report linking a regional and seasonal variation in an urban site as compared to a less-polluted site in Puerto Rico. We will attempt to isolate and identify strongly cytotoxic compounds from summer samples collected at Guaynabo—specifically from nonpolar and polar extracts. We also plan to evaluate heavy metal components in these extracts at Fajardo.

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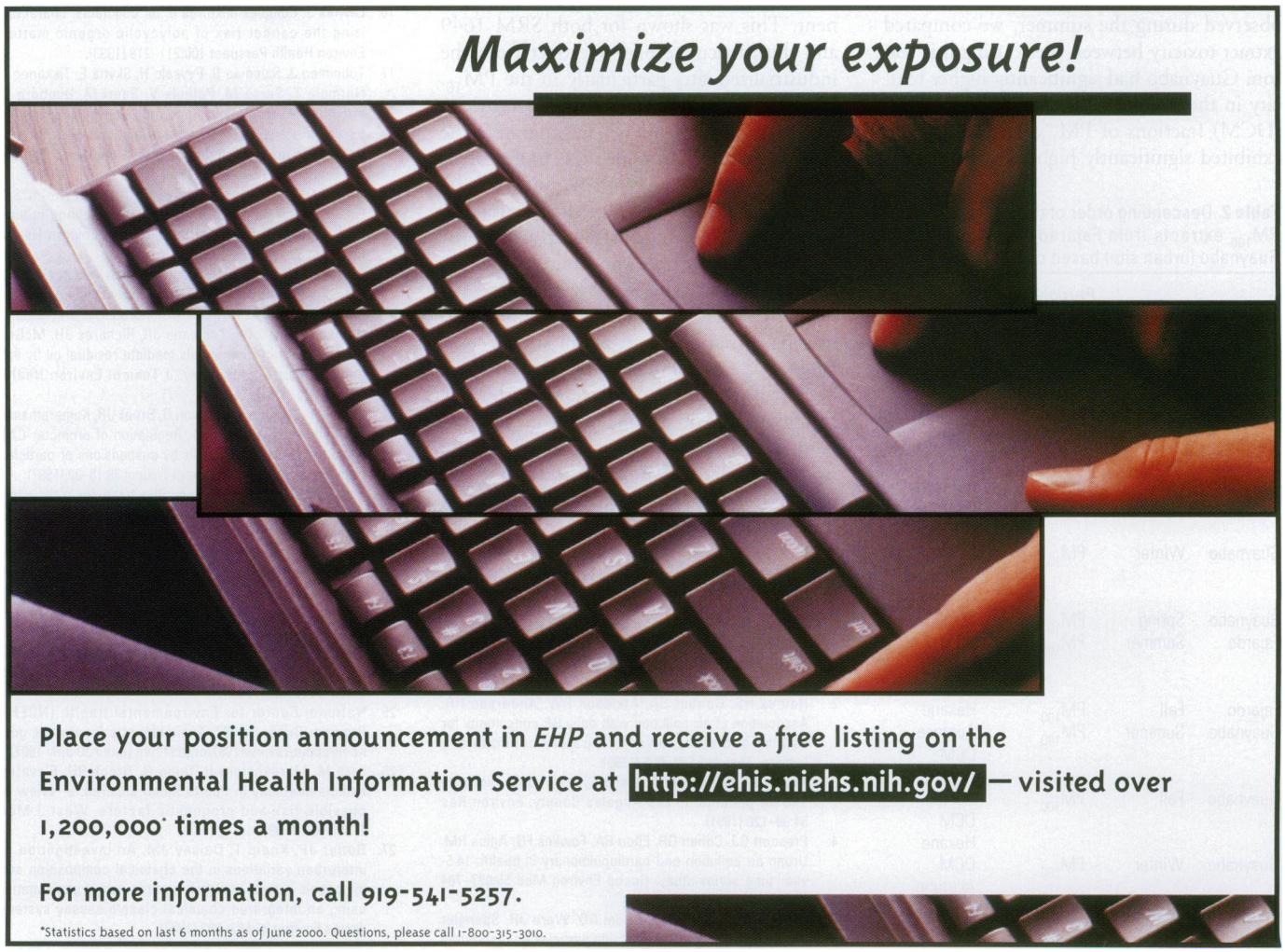
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