Role of chemical and biological constituents of PM$_{10}$ from Saharan Dust in the exacerbation of asthma in Puerto Rico

M. Ortiz-Martínez$^{1,2}$, E. Rivera-Ramírez$^{2,3}$, L. Méndez-Torres$^{1,2,4}$, and B.D. Jiménez-Vélez$^{1,2}$.

University of Puerto Rico-Medical Sciences Campus, Department of Biochemistry$^1$
Center for Environmental and Toxicological Research$^2$
San Juan PR, 00936
University of Puerto Rico-Río Piedras Campus, Department of Biology$^3$
University of California Irvine, Department of Microbiology and Molecular Genetics$^4$

Corresponding Author: Dr. Braulio D. Jiménez-Vélez

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ABSTRACT

Asthma is a chronic inflammatory disease of the airways whose prevalence in the US has increased 75% since 1980, particularly in children. Some environmental factors associated with increased asthma are: exposures to endotoxins (ENX) and other contaminants found in airborne particulate matter (PM). This research evaluates the role of chemical and biological constituents (such as ENX) present in PM$_{10}$ of Saharan dust in the exacerbation of pediatric asthma in PR. To accomplish this, we analyzed PM$_{10}$ for Fajardo and Guaynabo during 2004. We found that PM$_{10}$ levels increased when Saharan Dust Events (SDE) reached the shores of Puerto Rico. ENX content was measured and found to be higher in PM$_{10}$ Guaynabo aqueous extracts. Therefore, we evaluated whether SDE reaching PR correlated with an increase in asthma morbidity in children. A retrospective analysis of claims data for pediatric asthma cases, from the ASES (Health Insurance Administration of PR) database for the year 2004, revealed that the highest number of visits for both an urban (San Juan) and rural (Fajardo) site occurred in March. Coincidentally, March 2004 also contained the highest number of SDE reaching PR. We used an in-vitro model of lung epithelial cells to evaluate the cytotoxic and pro-inflammatory effects (IL-6 and IL-8 release) of SDE vs Non-SDE PM$_{10}$ in inland and Atlantic Ocean aqueous extracts. Results showed that the Guaynabo PM$_{10}$ extracts exerted a cytotoxic effect in a dose dependent manner and was more toxic than non-SDE PM$_{10}$ extract (p<0.05). Inhibition of ENX in extracts reduced its original cytotoxicity and pro-inflammatory effects.
INTRODUCTION

Asthma is a chronic inflammatory disease of the airways whose prevalence in the US has increased 75% since 1980, particularly in children (1). Both, the mainland and Puerto Rican children exhibit the highest asthma prevalence when compared to other minority or Latino subgroups in the US (2). The development and manifestation of this multifactorial disease depends on both genetic and environmental factors. Canino et al. (2006) proposed a conceptual model to explain asthma prevalence disparities in minority children (3). Some of the environmental factors that can be associated with increased asthma are: exposure to indoor allergens, viral infections, endotoxins (ENX), and pollutants, which are included in airborne particulate matter (PM). Epidemiological studies have found positive associations between PM and ENX levels and the exacerbation of asthmatic symptoms (4-6). Airborne PM$_{10}$ is a complex mixture of organic/inorganic compounds (within a size range) which, are considered to be “inhalable particles”, since they can be deposited in the lower part of the respiratory tract where they can trigger inflammation processes. However, the individual components responsible for their adverse effects have not been identified (7). PM$_{10}$ is composed of earth crust materials (like minerals), fugitive dust from roads, industries and domestic coal burning and biological materials such as bacteria and its components (like ENX), fungal spores, pollen fragments and leaf materials (8-9). In addition to these components originating from individual sites one can add those arriving from global influences of sources such as Dust Storms.

Puerto Rico is not exempt from these natural global inputs of dust. African dust originating from the Sahara and Sahel regions are the major sources of dust flux in the world with an estimated transport contribution of approximately 50-75% of the total estimate mass of 0.5-5 billion tons (10-11). African dust originating from the Sahara desert is transported across three main regions: Westward, across the Atlantic Ocean; Northward and Eastward into part of Europe and the Mediterranean Basin. The amount of dust transported across the Atlantic Ocean is the most voluminous, accounting for 30 to 50% of the total world output (12-13). This transport can take from 3-5 days to reach the Caribbean and the American Continent (10). The most impacted of all areas relies between latitudes 10º and 25ºN, affecting the Caribbean and the northeastern tip of South America and the USA. Areas impacted by desert dust such as the Caribbean are known to have some of the highest incidences of asthma worldwide (10). Long-term studies conducted in Barbados and Miami have shown that SDE contribute to increments of local PM levels (14). An increase number of bio-aerosols have also been reported when SDE impact the Caribbean region. Approximately half of the bacterial population isolated from SDE particles is Gram-negative, which contain ENXs in their cell walls (10).
Previous studies from our laboratory have reported regional and seasonal variations in ambient PM concentrations and composition in Puerto Rico (15-19). These variations are attributed to local anthropogenic sources and to natural events such as SDE and volcanic eruptions impacting the Caribbean region. A popular belief in PR is that SDE are associated with increased allergic and asthma attacks, however no systematic study has been conducted to confirm this belief. To our knowledge, only two groups have reported any association between SDE and the prevalence of asthma in the Caribbean (20-21). The first study, done in Barbados, found associations between the biogenic content in SDE and acute asthma symptoms. The second study, reported a significant association between increased pediatric asthma emergency room visits and SDE reaching Trinidad. However, there is another report that didn’t find any correlation between these variables (28).

Our laboratory has studied the pro-inflammatory effect of PM$_{2.5}$ aqueous extracts from Fajardo, PR and Guaynabo, PR in relation to IL-6, IL-8 and MCP-1 cytokines released by a human lung cell line, BEAS-2B (23). Other investigators have also studied cytokine release and mRNA expression (IL-6, between others) in BEAS-2B (human bronchial epithelial cells) in response to PM$_{2.5}$ and PM$_{10}$ extracts (7, 24). However, the possible toxicological and pro-inflammatory contribution of specific constituents such as ENX in Puerto Rican and Atlantic Ocean PM$_{10}$ containing SDE material has not been previously studied.

Therefore, the research presented here evaluates the PM$_{10}$ influx by SDE reaching Puerto Rico during the year 2004 and its possible contribution to the exacerbation asthma in Puerto Rican children. We approach this question by studying the effects of the various PM$_{10}$ SDE aqueous extracts on BEAS-2B cells response and their ENX content.

**MATERIALS AND METHODS**

**Retrospective Analysis** Aerosol index images were obtained from the Total Ozone Mapping Spectrometer (TOMS) for the year 2004 ([http://jwocky.gsfc.nasa.gov](http://jwocky.gsfc.nasa.gov)). PM$_{10}$ mass concentration data for that year were obtained for both municipalities (Fajardo/rural and Guaynabo/urban) from the Environmental Quality Board of Puerto Rico (EQB). Claims data for pediatric (0-18 years old) asthma cases for both municipalities were obtained from the Puerto Rico’s Health Insurance Administration (“ASES”). TOMS images were analyzed daily to identify SDE clouds reaching PR. The number of SDE per month was determined by matching TOMS images and PM$_{10}$ mass concentration (Table 1) at the sites. PM$_{10}$ data was compared to TOMS images in order to confirm the SDE. PM$_{10}$ concentration and claims data were organized by month and municipality and analyzed per days. Asthma cases were categorized by age.
Site Selection PM$_{10}$ (SDE and non-SDE) samples were collected at the EQB sampling station located at either municipality site: near the “Cabezas de San Juan” lighthouse at the northeastern shore of PR (Fajardo) and adjacent to a San Juan commercial port zone (Guaynabo). The EQB has designated Fajardo as a remote reference site to measure background levels of PM in Puerto Rico. We have selected Fajardo as a reference study site in order to minimize the contribution of local emissions from different combustion sources. PM$_{10}$ was also collected across the Atlantic Ocean in the Ronald H. Brown NOAA Research Vessel (AEROSE, 2004).

Preparation of PM$_{10}$ Aqueous Extracts PM$_{10}$ quartz filters were obtained from the EQB. Filters were extracted in HPLC grade water (Fisher) by sonicating the minced filter for 2 hrs. The extracts were dried after sonication using a Centrivap console (Labconco) and the respective mass determined gravimetrically (Table 2). Finally, the aqueous extracts were resuspended in water to a concentration of either 50 or 100 mg/ml. Two composites samples were prepared for each site (Fajardo, Guaynabo and Atlantic Ocean): one corresponding to SDE days (March of 2004) and other for Non-SDE days (March of 2004). All the extract composites were stored at -20˚C until further analyses. PM$_{10}$ aqueous extracts were classified as those derived from SDE and Non-SDE days (Table 2) as explained above. March was selected for the dose response experiments, because it showed the highest number of SDE and asthma cases during 2004.

Endotoxin Quantification Endotoxin concentrations in aqueous fractions of PM$_{10}$ were determined using the QCL-1000 Chromogenic Limulus Amebocyte Lysate Assay (Lonza Biosciences) as described by the manufacturer. Briefly, the assay is based on the activation of a proteolytic cascade by endotoxin in the serum of the horseshoe crab *Limulus polyphemus*. A colorless substrate peptide is added to the reaction and upon its proteolytic cleavage it releases p-nitroaniline, which absorbs at 405nm. Absorbance readings at 37˚C are recorded using a microplate reader (Ultramark microplate imaging system, Bio Rad, Hercules, CA). Plotting the absorbance change vs known endotoxin concentrations generates the standard calibration curve. The sensitivity for the assay is 0.1-1.0 EU/ml. Quantitative measurement of total ENX present in PM$_{10}$ collected at the Atlantic Ocean during AEROSE 2004 was determined with the kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay (Pyrochrome, Associates of Cape Cod) following the manufacturer’s instructions. The detection limit for this assay is 0.005 EU/ml.

Cell Exposures Human bronchial epithelial cells (BEAS-2B, ATCC) were cultured and maintained with Keratinocyte Growth Medium (KGM-2, Lonza Bioscience) at 37˚C, 5% CO$_2$. The cells were grown in 96-well plates until reaching a confluence of 80-90%. Cells were
exposed to different PM$_{10}$ aqueous extracts concentrations for 24 hrs. Controls were verified in three different ways: unexposed (media alone), dilution (water used in the aqueous extracts) and process (aqueous extract derived from a blank filter). Endotoxin inhibition experiments were conducted by pre-incubating cells to 10µg/mL of either Polymyxin B sulfate (PMB) or recombinant endotoxin-neutralizing protein (ENP, Associates of Cape Cod) in a sonic water bath for 30 min prior to cell treatment. Appropriate controls were exposed to media pre-incubated with the ENX inhibitors, LPS and LPS pre-treated with the inhibitors. Cell supernatants were collected after each exposure and stored at -80°C until further cytokine analyses.

**Cell Cytotoxicity** PM$_{10}$ cytotoxic effects on exposed cells were assessed using the neutral red bioassay (Sigma, St. Louis, MO), which is based in the capacity of viable cells to actively uptake the neutral red dye and retain it in lysosomes. Triton X-100 (25µg/ml) was used as a positive control of cell cytotoxicity.

**Cytokine Assay** The levels of pro-inflammatory cytokines (IL-6 and IL-8) in cell supernatants were determined simultaneously using a multiplex bead assay (Millipore) in the dual laser flow analyzer Luminex 100 (Luminex Corp., Austin, TX). Multiplex bead assays are solid phase immunoassays that allow simultaneous quantitative detection theoretically of up to a 100 analytes in a single microtiter by flow cytometry. Briefly, 40µl of each sample were added to a 96-well filter plate and incubated overnight at 4°C with 25µl of the antibody-coupled beads. Unbound proteins were washed away before the addition of 25µl biotinylated detection antibody. After 1hr of incubation with the detection antibody, the plates were washed and 25µl of streptavidin-phycoerythrin detection solution were added. The median fluorescence intensities of fluorochrome-conjugated antibodies bound to individual microspheres were derived from the flow analysis of 100 microspheres per well (in duplicate). Standard curves for each cytokine were plotted using a 5-parameter logistic fit.

**Statistical Analyses** To assess differences among groups an ANOVA statistical assay was performed followed by the Tukey test for multiple comparisons. The criterion for statistical significance was set at p≤0.05. Statistical analyses were performed using the GraphPad InStat 3 software.
RESULTS

Retrospective analysis

A retrospective analysis was performed for the year 2004 for three types of data: 1) aerosol index (AI) images obtained from the Total Ozone Mapping Spectrometer (TOMS), 2) PM$_{10}$ mass concentration and 3) pediatric asthma reported cases. The AI images showed SDE reaching Puerto Rico at specific dates often during the spring and summer seasons (Figure 1). The specific days that can be attributed to those events are listed in Table 1. The months of March, May and August contained the highest number of SDE, followed by June and July (Table 1). The rest of the months showed very little SDE activity (January and October) or nothing at all (Figure 1).

PM$_{10}$ ($\mu$g/m$^3$) mass concentration data showed a seasonal variation pattern for both, the rural and urban site. Both sites exhibited high levels of PM$_{10}$ during the month of March (spring) and June (summer), which correlates with the temporal SDE pattern detected by TOMS. As expected, the PM levels at the urban site were higher than those at the rural site (Figure 2).

The “ASES” data showed higher number of asthma cases for children of $\leq$5 yrs of age (Figure 3). These observations were similar for the two municipalities analyzed: Fajardo (rural site) and San Juan (urban site). The month with the highest number of cases for children of $\leq$5 yrs of age was March, which corresponds to one of the months with the most SDE reaching the island. An inverse relationship was observed between monthly PM$_{10}$ levels and asthma cases during 2004, where PM$_{10}$ levels increase during March and June and asthma cases increase during March and start decreasing during the summer until a minimum (June-September). This behavior was similar for both municipalities. It is interesting to notice a relatively high number of cases in the rural area (also noticed to a lesser extent in the urban area) during the months of November to February (end of fall and winter seasons).

Dose response experiments – Aqueous Extracts

The aqueous extracts from the rural site were not toxic at any concentration and only exerted a slight decrease in cell viability at the highest SDE and Non-SDE PM$_{10}$ dose of 250$\mu$g/ml (Figure 4A). SDE aqueous extracts from the urban site (Figure 4B) exhibited a dose dependent cytotoxic effect ($R^2=0.962$). Both Non-SDE extracts doses used exhibited cell cytotoxicity of approximately 18%, being one reduction statistically different from the medium. In addition, a significant difference between SDE and Non-SDE extracts was observed at the highest dose. In general, the urban aqueous extracts were more cytotoxic than the rural extracts.
Due to the limitation of Atlantic Ocean PM$_{10}$ material, we only tested aqueous extracts cytotoxicity at 50µg/ml. No cytotoxicity was observed at this dose (Figure 5).

**Endotoxin concentrations and inhibition**

The amount of ENX (EU/m$^3$) in PM$_{10}$ collected within the Saharan storm in the Atlantic Ocean (March 2004) was approximately 13 folds higher than the normal background levels at sea in a Non-SDE day (Table 3). ENX concentration (EU/mg PM$_{10}$) in the aqueous extracts obtained from the SDE PM$_{10}$ was only twice as high as the respective Non-SDE extract (Table 4). However, when SDE reaches the island the amount of ENX recovered in the aqueous extracts appears not to be reflected in the aqueous fraction at either site (rural or urban) since SDE and Non-SDE ENX concentrations remain practically unaltered (Table 4) except with a slight increase in the SDE from the urban sample. Pre-treatment of aqueous extracts with ENX inhibitors, PMB and ENP reverted the cytotoxic effects exerted by the PM$_{10}$ aqueous extracts (Figure 6). The inhibitors effect was statistically significant only for the extracts from the urban site, where PMB increased cell viability ~10% and ENP exhibited a more potent effect (Figure 6B).

**Cytokine Secretion**

Preliminary experiments using PM$_{10}$ aqueous extracts from the rural site provoked no significant effect on IL-6 or IL-8 cell secretions as compared to the cytokine concentrations in the controls (Data not shown). No significant difference was found in cytokine secretion between extracts with or without the ENX inhibitor (PMB). The urban aqueous extracts showed a slight IL-6 and IL-8 induction effect (Data not shown). Aqueous extracts obtained from the urban site exhibited higher IL-6 secretion effect as compared to the rural extract. This inductive effect of aqueous extracts at 50µg/ml was partially suppressed with PMB. The SDE urban extract exhibited significantly higher IL-8 inductive effect than its counterpart Non-SDE sample. The SDE urban extract induced approximately 25pg/ml more IL-8 than the Non-SDE extract. The 50µg/ml concentration from the Atlantic Ocean SDE extract showed a significant induction effect on IL-6 secretion (Figure 7A) while the Non-SDE did not exhibit any effect on IL-6. Interestingly this induction on IL-6 production disappeared upon pre-treatment of the SDE extract with PMB. However, IL-8 release wasn’t induced at the same dose (Figure 7B).
DISCUSSION

The use of AI images (TOMS) allowed the identification of the SDE cloud as it reached the shores of Puerto Rico during specific dates in 2004. This satellite data used in conjunction with the specific ground PM$_{10}$ data measurements collected by the EQB sampling network were essential for the SDE and Non-SDE composite extracts designation. The outcome of this established protocol classified March and June as months of the most abundant SDE. Baring in mind that Caribbean region receives a high input of Saharan Dust and using the outcome of the retrospective analysis of 2004 it was possible to establish March as a month that is high in both asthma cases in children and high dust parameters. This is in agreement with what was reported for the Caribbean island of Trinidad in 2001-2002 (21). Increased pediatric asthma admissions were associated with increased Saharan Dust cover during that year. However, in our analyses the month of June although high in dust storm particles it failed to show the concurrent increase in the number of pediatric asthma admissions. The difference observed between March and June could be explained by various factors such as the difference between SDE number, stability, composition; and length of cloud exposure days. The high number of cases found during the winter months (where there are no SDE/lower PM$_{10}$ mass concentration) could be associated to other biological or chemical components in the SDE such as fungal spores, which increases during months characterized by high humidity. Muñoz-López (2004) also states that climatic changes (such as humidity changes) have an effect on fungal sporulation and is related to the incidence of asthma and other respiratory diseases.

Asthma prevalence in Puerto Rico has increased a lot in recent years, particularly in children. The Center for Disease Control and Prevention (CDC) reported a current overall asthma prevalence of 11.6% for Puerto Rico. For the year 2004, the president of the Society of Pneumology, Juan Jiménez affirmed that asthma prevalence in the US was 5-10%, while in Puerto Rico it was 11-16% (25). He reported an annual mortality rate of 160 due to asthma in PR. Both, island and USA mainland Puerto Ricans have high asthma prevalence. A recent article in “Medicina y Salud Pública” reports that Puerto Rico has mortality due to asthma that is twice as high as in the USA (26). Ledogar et al (2000) found that Puerto Ricans living in New York City had asthma prevalence very similar to those living in the island (~16-18%), especially pediatric patients from 0-12 yrs of age. A possible asthma genetic trait in Puerto Ricans is suggested since the prevalence is consistently high in different environments. Other researchers have also proposed the possibility of a gene-environment interaction influencing asthma in Puerto Rico. Choudry et al (2004) found a gene environment interaction between CD14 (co-receptor of the Toll-like receptor 4, TLR signaling pathway, which is activated by lipopolysaccharide, LPS or ENX) genotypes and Environmental Tobacco Smoke (ETS) in a
Puerto Rican population of 365 of 10-15 yrs of age, living in San Juan. Here we report ENX in PM\textsubscript{10} extracts from SDE as a possible environmental factor that is a potential candidate for a gene-environment interaction that could change asthma development in Puerto Ricans.

Aqueous extracts of PM\textsubscript{10} from SDE at the urban site exhibit a strong dose-dependent cytotoxic relationship. These results clearly suggest that some chemical (such as minerals) or biological (such as ENX) constituents in the aqueous extracts from Saharan dust PM\textsubscript{10} are toxic to human lung epithelial cells (BEAS-2B). This type of cells are found in respiratory airways and are sentinels to chemical and biological exposure preceding inflammatory responses that can eventually lead to respiratory distress such as asthma. Therefore, PM\textsubscript{10} is a possible source of chemical and biological compounds that can influence the exacerbation of asthma in PR.

We have found that ENX concentrations are higher in whole extracts from PM\textsubscript{10} obtained during a SDE collected at the Atlantic Ocean. The amount of ENX at the edge of the storm is about 12 fold higher than what is found at background levels. This is evidence of the high levels of gram-negative bacteria associated with African dust (10). As far as we know this is the first study that reports the presence of ENX in Saharan Dust. The ENX concentrations in aqueous extracts from the urban site are about 7 fold higher than those from the rural site. Furthermore, ENX in SDE PM\textsubscript{10} were determined to be an important constituent to cytotoxicity. This was confirmed by the use of 2 specific ENX inhibitors: Polymyxin B and Endotoxin-neutralizing protein (ENP). Both inhibitors increased cell viability upon treatment of SDE aqueous extracts from the urban site. This phenomenon was not observed with either of the rural extracts suggesting that Saharan dust in association with other constituents found at the urban site is causing cell cytotoxicity. Prospero et al (2008) confirms that there may be subtle changes between dust and asthma and suggests a link between African dust and anthropogenic factors in Barbados. Prospero et al (2001) also suggests that African dust in conjunction with emissions from local and regional sources could exceed the US Environmental Protection Agency’s PM\textsubscript{10} standards.

It has been shown that ENX is a potent inflammatory agent that can cause chronic airway disease; consequently it is logical to consider its role in the exacerbation of asthma (30). The inflammation process involves the release of a number of cytokines that mediates antigen processing and the regeneration of damaged tissue. IL-6 and IL-8 are classical cytokines related to PM\textsubscript{10} in vitro effects on BEAS-2B and alveolar macrophages cells (7, 31-34). Our study found that SDE PM\textsubscript{10} aqueous extracts from the urban site induced the release of IL-6 and IL-8, indicating a possible role of these extracts on bronchial epithelial cells inflammation. IL-6 is one of the most important players in acute inflammation of lung cells, and one of the primary pro-inflammatory cytokines induced in response to lung insults (35). IL-8 is the major chemo-attractant of human neutrophils (36). Both of these cytokines are key players in promoting the
immune response against PM$_{10}$ extracts as a respiratory insult. The induction of IL-8 was found to decrease upon PMB pre-treatment of SDE Atlantic Ocean aqueous extract, suggesting an ENX-dependent effect on cytokine release. Other researchers have reported cytokine reduction after treatment of PM$_{10}$ using PMB, using human alveolar macrophages (34). However, both cell types are found in the lungs and can release cytokines that initiate an inflammatory cascade in response to ENX (37).

Summarizing, our research implicates the environmental component of ENX found in PM$_{10}$ from SDE as a possible cause of asthma exacerbation in Puerto Rican children, which are highly exposed to Saharan dust during certain months of the year (such as March 2004). We demonstrated that an ocean dust storm formed during the month of March off the African coast was very rich in ENX. The combination of ENX in Saharan dust with anthropogenic sources (like those found at the Guaynabo urban site) together with genetic predisposition could explain the high incidence of asthma, particularly in Puerto Rican children. Further studies are needed to evaluate the possible contribution of gene-environment interactions between SDE PM$_{10}$ and polymorphisms of the ENX receptors (Toll-like receptors, TLR’s) in Puerto Rican asthmatic children. In the long range this research could be valuable for understanding some causes and mechanisms of action of compounds associated with the disease and hence in the re-evaluation of current targets for drug therapy.

**ACKNOWLEDGEMENTS**

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ABBREVIATIONS

ENX = Endotoxins
SDE = Saharan Dust Events, African Dust, presence of
Non-SDE = Non-Saharan Dust Events, absence of
PMB = Polymyxin B Sulfate
ENP = Endotoxin-Neutralizing Protein
EU = Endotoxin units

REFERENCES


**FIGURES**

**Figure 1:** Total Ozone Mapping Spectrometer (TOMS) images for January, March, June and October of 2004. Aerosol Index is represented by the colors grey, yellow and red. The red color denotes a higher intensity. Images were obtained from the NASA website [http://jwacky.gsfc.nasa.gov](http://jwacky.gsfc.nasa.gov)
Figure 2: Mean $PM_{10}$ Mass Concentration for Guaynabo (urban) and Fajardo (rural) during 2004. Data obtained from the EQB of Puerto Rico.
**Figure 3:** Pediatric Asthma Cases reported by the Health Insurance Administration of Puerto Rico ("ASES") in *A:* Fajardo and *B:* San Juan during 2004. The data is presented in three categories: 0-5, 6-12 and 13-18 years old.
Figure 4: BEAS-2B Cytotoxicity to SDE/Non-SDE PM$_{10}$ Aqueous Extracts from A: Fajardo and B: Guaynabo during March 2004. A value <80% was considered cytotoxic. Bars represent means ± SEM, n=3.
Figure 5: BEAS-2B Cytotoxicity to SDE/Non-SDE Aqueous Extracts of PM$_{10}$ collected across the Atlantic Ocean (AEROSE, March 2004). A value $<80\%$ was considered cytotoxic. Bars represent means ± SEM, $n=3$. 
Figure 6: BEAS-2B Cytotoxicity to SDE PM$_{10}$ Aqueous Extracts from A: Fajardo and B: Guayanabo in the presence of ENX Inhibitors. A value <80% was considered cytotoxic. PMB stands for Polymyxin B sulfate and ENP for Endotoxin-neutralizing protein. Bars represent means ± SEM, ***p<0.001, **p<0.01, n=3.
Figure 7: Induction of A: IL-6 and B: IL-8 in BEAS-2B exposed to PM$_{10}$ Aqueous Extracts from the Atlantic Ocean (AEROSE, March 2004). Bars represent means ± SEM, *p<0.05, n=3.
### TABLES

**Table 1: Number of Saharan Dust Events (SDE) for the year 2004**

<table>
<thead>
<tr>
<th>Month</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
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</thead>
<tbody>
<tr>
<td>SDE</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Event Dates*</td>
<td>1,12,16,22,26</td>
<td>1,16,21</td>
<td>2,12,16,25,31</td>
<td>1,15,19,23</td>
<td>3,8,15,20</td>
<td>1,6,10,16,21</td>
<td>9,21</td>
</tr>
</tbody>
</table>

* The event dates were determined by a peak of PM$_{10}$ mass concentration, SDE length range was 3-8 days.

**Table 2: March 2004 PM$_{10}$ Aqueous Extracts Mass (mg)**

<table>
<thead>
<tr>
<th></th>
<th>SDE</th>
<th>Non-SDE</th>
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</thead>
<tbody>
<tr>
<td>Fajardo</td>
<td>68.2</td>
<td>11.4</td>
</tr>
<tr>
<td>(Rural)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guaynabo</td>
<td>43.0</td>
<td>12.4</td>
</tr>
<tr>
<td>(Urban)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic</td>
<td>0.21</td>
<td>0.36</td>
</tr>
<tr>
<td>Ocean</td>
<td></td>
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</table>

**Table 3: ENX concentration in PM$_{10}$ collected across the Atlantic Ocean (March 2004)**

<table>
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<tr>
<th>Extract</th>
<th>Date</th>
<th>EU/m$^3$</th>
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<td>Pre-SDE</td>
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<td>0.50</td>
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<td>SDE</td>
<td>3/7/04</td>
<td>6.41</td>
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**Table 4:** Inland and Ocean $ENX$ concentration in $PM_{10}$ Aqueous Extracts from March 2004

<table>
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<tr>
<th>Locations</th>
<th>Extracts</th>
<th>EU/mg PM$_{10}$</th>
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<tbody>
<tr>
<td>Inland</td>
<td>SDE (Rural)</td>
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<tr>
<td></td>
<td>Non-SDE (Rural)</td>
<td>0.32</td>
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<tr>
<td></td>
<td>SDE (Urban)</td>
<td>2.04</td>
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<tr>
<td></td>
<td>Non-SDE (Urban)</td>
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<td>Non-SDE</td>
<td>0.16</td>
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