Ah RECEPTOR MEDIATED EFFECTS OF PARTICULATE ORGANIC EXTRACTS FROM THE PASO DEL NORTE AIRSHED ALONG THE U.S.-MEXICO BORDER

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Introduction

Particulate matter (PM) is comprised of organic and elemental carbon aggregates containing various metals, acid salts, and organic pollutants[1]. Organic compounds adsorbed to PM can contain carcinogens and mutagens[2]. Recent epidemiological studies have demonstrated causal associations between exposure to particulate matter and an increased risk of lung cancer and adverse affects on fetal development[3]. Studies conducted in many cities around the world consistently demonstrate mutagenicity of PM co-pollutants[4,5]. The Paso del Norte air basin encompass three cities along the U.S.-Mexico border; El Paso, TX, Sunland Park, NM, and Ciudad Juarez, Chihuahua, Mexico. In the Paso del Norte air basin, PM_{10} levels exceed federal guidelines, therefore increasing the risk of human exposure to harmful environmental compounds. Anthropogenic and man-made natural sources of contaminants from both sides of the border contribute to air pollution within this region. Sources of point and non-point pollutants have been identified as vehicular traffic (about 20%), open burning of scrap fuel during winter (40%), brick kilns, unpaved roads in Juarez (50%), and two refineries (J. Serna, Physical Science Laboratory, NMSU, pers comm.). In addition, winter inversion layers trap particulate matter in the valley and increase PM_{10} levels.

The specific aim of this study is to identify biomarkers which would serve as an indicator of air toxics in the Paso del Norte airshed. This study will provide baseline data on biological alterations associated with exposure to various types of organic contaminants and aid in the identification of important environmental contaminants.

Materials and Methods

PM_{10} filters were collected during the winter of 1998-1999 from high volume air samplers from two sites in El Paso and one site in Juarez. The sites selected in El Paso were Northeast Clinic, reference site; and Tillman Health Center, a site with historically high levels of PM_{10} in downtown. The site selected in Juarez was Advance Transformer located next to a transformer production facility and within 200 yards of a brickmaking district. Fuels used in the brick kilns includes sawdust, wood pallets, tires, and other scrap fuel. One-half of the 8" x 10" filters were extracted individually using a Soxhlet apparatus with 100 ml of dichloromethane (DCM) for 24 h, followed by concentration with a Kuderna-Danish apparatus (EPA method 3450C). Samples were stored in DCM in amber vials with PTFE-lined caps at 4°C[6].

Air equivalents (AE)are defined as the amount of respirable air an average adult breathes in a specified time period. An average individual exchanges approximately 22-24 m^3/day[7]. Measurement of CYP1A1 activity was measured in H4IIE cells cultured according to Sanderson et. al.[8] and exposed various amounts of organic extracts resuspended in DMSO (final concentration

0.5%). Following exposures, cells were rinsed with Hanks balanced salt solution, lysed with mammalian protein extraction reagent (Pierce, Rockford, IL), homogenized and centrifuged at 160 x g for 10 minutes at 4°C. Supernatants were collected and stored in buffer with 20%glycerol at -20°C. Ethoxyresorufin O-deethylase (EROD) activity was measured fluormetrically.

Because compounds present in organic extracts might inhibit EROD activity[6], we also used the chemically activated luceriferase expression (CALUX) bioassay to evaluate PM samples. Methods for culturing recombinant mouse cells and measuring luciferase activity followed Ziccardi et. al.[9].

To assess toxicity of extracts over a range of air equivalents, XTT assays were performed. Both HII4E and recombinant mouse hepatoma cells were tested with 1 and 10 minutes of air equivalents. Cells were measured spectrophotometrically at 450 nm to determine cell viability.

Results and Discussion

Dose response relationships were determined for PM extracts and EROD and luciferase induction. For P4501A1 activity in HII4E cells, we observed highest activity at 12 minutes of air equivalents, after which inhibition was noted with extracts from sites with higher particulates. In contrast, for the AhR-luc reporter system, the response remained linear between 30 seconds and 90 minutes of air equivalents.

Cytotoxicity assays were performed to ensure that the air equivalent dose used in the experiments does not cause overt toxicity. We found no cytotoxicity in HII4E cells exposed to 10 minutes air equivalents. Cytotoxicity measurements will be made in recombinant mouse cells exposed to 1 minute AE.

The highest EROD activity was observed in cells exposed to extracts from the Advance site. The Advance PM sampling site is situated in Ciudad Juarez within a maquiladora district immediately adjacent to a brickmaking area. Brickmakers are known to use a variety of scrap fuels to fire bricks, including tires, sawdust from industrial facilities, woods, and other scrap material. An initial analysis of chemicals contained in the DCM extract show that the PCB concentration at the Advance site is approximately 1.1 ng/m^3 (W. Jarman, U. of Utah, pers. comm.). We observed similar patterns between EROD activity and amount of particulates collected from any one date. This suggests that the composition of PM remains fairly constant, regardless of the amount of particulates collected.

EROD activity associated with the Northeast and Tillman sites was reduced compared to Advance, suggesting either lesser amounts of AhR ligands or inhibition by those chemicals. Data from the AhR luc assay followed the same pattern as EROD data for all dates, suggesting a lesser amount of ligands present. However, at the Advance site, luc activity was significantly induced on Jan 30 whereas EROD activity was moderate (8 pmol/min/mg protein) relative to other dates. This suggests that significant amounts of Ah receptor ligands acted to inhibit EROD activity.

We examined the relationship between total particulates (g) and the activity of the two biomarkers to determine if they varied in the same fashion. On most dates, when particulate loads were high, both EROD and LUC activity increased. However, in some instances this was not the case. For example, on January 30 at the Advance site, PM levels were modest, 0.15 g per filter, EROD activity was modest (8 pmol/min/mg prot) whereas LUC activity was over 3500 RLU/mg protein. This would suggest that there are increased amount of AhR ligands probably associated with scrap fuel used in the brick kilns that are inhibiting EROD activity. Further, fuel composition probably varies on different dates which accounts for the discrepancy between PM weight and LUC activity.

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Gravimetric data (g) per filter for January, 1999 from 3 sites in the Paso del Norte airshed

Date	Advance	Northeast	Tillman
1-6-99	.276	.07	.101
1-12-99	.111	.06	.057
1-18-99	.152	.031	.047
1-24-99	.203	.043	.087
1-30-99	.163	.04	.043