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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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1 Short running head: Biological activity of PM₁₀ extracts

2

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5

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16 Abbreviations:

17 PM₁₀ particulates less than 10 microns in diameter

18 POM particulate organic matter

19 PAHs polycyclic aromatic hydrocarbons

20 PCBs polychlorinated Biphenyls

21 TCDD 2,3,7,8 -tetrachlorodibenzo-p-dioxin

22 PCDDs polychlorinated dibenzo-p-dioxins

23 AhR aryl hydrocarbon receptor

24 DCM dichloromethane

25 EROD ethoxyresorufin-O-deethylase

- 1 BCA bicincholinic acid
- 2 RLU relative light units
- 3 BNF β -naphthoflavone
- 4 BaP benzo[a]pyrene
- 5 GC/MS gas chromatography/mass spectrometry
- 6

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1 **ABSTRACT**

2 Biological activity of dichloromethane-extracted PM₁₀ obtained from filters at three sites
3 in the Paso del Norte airshed, composed of El Paso, TX and Juarez, Chihuahua, Mexico, and
4 Sunland Park, NM, was determined. The extracts were rich in polycyclic aromatic hydrocarbons
5 (PAHs) and had significant biological activity, measured using two *in vitro* assay systems:
6 ethoxyresorufin-O-deethylase (EROD) induction and the AhR-receptor luciferase reporter
7 system. In most cases, both EROD (5.25 pmol/min/mg protein) and luciferase activities (994
8 RLU/mg) were highest in extracts from the Advance site, located in an industrial neighborhood
9 in Juarez. These values represented 58% and 55 % respectively of induction associated with 1
10 μM beta-naphthoflavone exposures. In contrast, little activity was observed at Northeast, the
11 reference site. In most cases, luciferase and EROD activity from extracts collected from
12 Tillman, situated in downtown El Paso, fell between those observed at the other 2 sites. Overall,
13 a statistically significant correlation existed between PM₁₀ and EROD and luciferase activities.
14 Chemical analysis of extracts collected from Advance demonstrated concentrations of most
15 PAHs were higher than those reported in most other metropolitan areas in the United States.
16 Calculations made with this data suggest a cancer risk of 5 –12 cases per 100,000 people. This
17 risk estimate as well as comparisons with the work of other investigators raises concern
18 regarding the potential for adverse health effects to the residents of this airshed. Further work is
19 needed to understand the sources, exposure, and effects of PM₁₀ and POM in the Paso del Norte
20 airshed.

21

1 INTRODUCTION

2 The Paso del Norte airshed is comprised of a basin formed by mountains that surround El
3 Paso, TX and Sunland Park, NM in the United States and Ciudad Juárez, Chihuahua in Mexico.
4 With a population over 2 million, it is one of the largest metropolitan areas along the border.
5 Visibility in the Paso del Norte airshed is frequently poor, especially in winter, and respiratory
6 problems are common (Barron 1999). El Paso has been designated as a federal non-attainment
7 area, associated with ozone and particulates (PM₁₀) exceedances. Recently, an index was
8 developed to reflect long-term exposure to air pollutants (Kyle et al. 2002). U.S. cities were
9 ranked according to a weighted estimate of exposure to criteria air pollutants; El Paso was
10 ranked 6th worst in the nation, following Los Angeles, Phoenix, Riverside, Orange County, and
11 New York City.

12 Sources of PM₁₀ in the Paso del Norte airshed share some similarities with other
13 urbanized areas, but some aspects of emissions and climatology are unique to this region. High
14 ambient PM₁₀ levels result from a wide range of emission sources and their presence in air is
15 affected by meteorological conditions, in particular strong inversions trap PM₁₀ in the winter.
16 Emissions are particularly high in the Paso del Norte airshed because of the high percentage of
17 older vehicles, many without catalytic converters; a significant amount of diesel exhaust
18 associated with NAFTA-related truck traffic at U.S.-Mexico border crossings; and the use of
19 wood, tires, and other scrap fuels for both residential heating and the firing of bricks. These
20 sources of PM₁₀ are known to produce particulate organic material (POM) mixtures of thousand
21 of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated
22 biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins (PCDD), many of which are highly
23 toxic and carcinogenic (Cuppitt et al. 1994; DeVito and Birnbaum 1995). Numerous
24 epidemiological studies have shown an association between ambient air particulates and
25 increased morbidity and mortality (Folinsbee 1992; Pereria et al. 1998; Schwartz 1994). Recent

1 studies have shown that ultrafine particles may play an important role in cardiopulmonary
2 diseases (Pope et al. 2002). The mechanism by which PM causes these adverse effects is the
3 topic of significant study (Harrison and Jianxin 2000). PAHs, including nitro-derivatives,
4 account for approximately 80% of mutagenic activity in urban PM extracts (Alsberg et al. 1985;
5 Harrison and Jianxin 2000). In addition to their mutagenicity, PAHs can interfere with certain
6 developmental processes and nourishment of the fetus (Sram 1999). Environmental exposure to
7 PAHs in heavily polluted areas such as Krakow, Poland has caused increased levels of white
8 blood cell PAH-DNA adducts in both mothers and infants. In this study, our goals were to
9 identify the presence of these potential harmful PAHs in POM by using two *in vitro* assays, to
10 quantify levels of selected PAHs, and, to the degree possible, relate these findings to the risk of
11 adverse health effects.

12

1 MATERIALS AND METHODS

2 Site selection and sample collection of PM₁₀ filters

3 PM₁₀ samples were collected on glass fiber filters using high volume air samplers (1400
4 m³/24 hour) during the winter of 1998-1999 from two sites in El Paso and one site in Juarez.
5 The sites selected in El Paso were Northeast Clinic, a site in a residential neighborhood about 1
6 mile from the desert; and Tillman Health Center, a site with high levels of PM₁₀ in downtown El
7 Paso. The Northeast site was selected as the reference site based on gravimetric data indicating
8 PM₁₀ levels are the lowest in the region. The site selected in Juarez was Advance Transformer,
9 located next to a transformer production facility in an area of maquiladoras and within 200 yards
10 of a brickmaking district. Brickmakers typically use scrap fuels such as sawdust, tires, and
11 lumber. The El Paso City County Health and Environmental District and Departamento de
12 Ecologia, Cd. Juárez were responsible for setting up the filters, collecting them after the 24 hour
13 monitoring period as well as recording gravimetric measurements. PM₁₀ filters were then
14 collected from the local agencies one week after the PM₁₀ monitoring date. During this period,
15 filters were protected from light and moisture. Using latex gloves, each filter was cut into a 5"x
16 8" rectangle with ethanol-rinsed scissors, wrapped in aluminum, labeled, and stored at -20°C in a
17 dessicator until further processing.

18 Extraction of organic material

19 All glassware used for organic extractions and storage was washed with Alconox
20 detergent for 20 minutes, followed by rinsing with distilled water, and acid wash in 1 N nitric
21 acid (ACS certified plus) for one hour. Glassware was then rinsed with distilled water followed
22 by rinsing with dichloromethane (DCM, Optima, Fisher Scientific, Pittsburgh, PA) and baked for
23 one hour at 100°C. One-half of an 8" x 10" filter was extracted individually using a Soxhlet
24 apparatus with 100 ml of DCM for 24 h, followed by concentration of the extract with a

1 Kuderna-Danish apparatus per EPA Method 3450C (Legzdins et al. 1995). DCM was used for
2 extraction because it effectively extracts broad classes of organics including those that are
3 mutagenic such as PAHs (Hannigan et al. 1994; Nielsen 1992). Samples were concentrated to a
4 volume of approximately 1 ml and stored in amber vials with PTFE-lined caps at 4°C. Prior to
5 use of extracts for experiments, DCM was evaporated under a gentle stream of nitrogen in a N-
6 VAP (Organomation, Berlin, MA) and the extracts were re-suspended in dimethyl sulfoxide
7 (DMSO, cell culture grade, Sigma, St. Louis, MO). In addition, glass fiber thimbles (Schleicher
8 & Schuell, Keene, NH) and three clean PM₁₀ glass fiber filters (5"x 8") were extracted as
9 described previously and tested as controls/blanks using EROD and luciferase assays.

10 Cell Culture

11 Rat hepatoma (H4IIE) cells were used to measure CYP1A1-related enzyme activity (i.e.,
12 ethoxyresorufin O-deethylase (EROD). Cells were purchased from the American Type Cell
13 Culture (Marassas, VA) and cultured in Minimum Essential Medium (MEM), Eagle with Earle
14 Salts, with 7.5% fetal bovine serum and 7.5 % newborn calf serum with 50 µg/ml gentamicin
15 sulfate at pH of 7.4. H4IIE cells were grown at 37°C in a humidified incubator with a 5% CO₂
16 atmosphere and sub-cultured weekly by dissociating with 0.25% (w/v) trypsin and 1 mM EDTA
17 in calcium and magnesium-free Hanks buffer, pH 7.4 (Sigma, St Louis, MO).

18 Recombinant mouse hepatoma (H1L1.c2) cells were used to determine the presence and
19 relative activity of AhR ligands in the chemical extract. H1L1.c2 cells contain a stable
20 integrated reporter plasmid (pGudLuc1.1) that responds to TCDD-like chemicals with the
21 induction of luciferase enzyme (Garrison et al. 1996). H1L1.c2 cells were cultured in MEM α
22 with L-glutamine, 10% fetal bovine serum, and 1% penicillin-streptomycin at pH 7.4 and grown
23 at 37°C in a humidified incubator with 5% CO₂ and sub-cultured bi-weekly.

24

1 Analytical Methods

2 *Range-finding*

3 The selection of concentrations for cell exposure for experiments was based on an
4 analysis of the response of both H114E and H1L1.c2 cells to various volumes of extracts. To
5 identify physiologically-relevant amounts of the DCM extracts to use, we estimated what an
6 average adult male might be exposed to in a 24 h period. A value of 20 m³/day was the default
7 selected for the ventilation rate for an adult male. Seventy percent of this total, or 14 m³ /day,
8 was the estimate used for the alveolar ventilation rate, assuming 100% absorption. Finally, the
9 default collection rate of 1400 m³/24 h for Hi-Vol PM₁₀ sampler was used. To obtain the
10 relationship between m³ air and μl DCM extract, we first divided 1400 m³ (air thru Hi-Vol
11 sampler in 24 h) by 14 m³ (alveolar air inhaled in 24 h). This quotient was then divided by the
12 total μl DCM extract for each sample. The dose range used for the EROD range finder was
13 based on our estimate of the volume of air an adult male would breathe in 3 – 1050 minutes,
14 equal to 0.030 – 10.21 m³. H4IIE cells were seeded at 450,000 cells/well and, after 24 h, were
15 incubated for an additional 24 h with 15 μl (0.5% of total volume of cell culture media) of the
16 DCM extract. Following exposures, cells were rinsed with Hanks buffer, and lysed with
17 mammalian protein extraction reagent (M-PER™, Pierce, Rockford, IL). Homogenates were
18 then centrifuged at 160 x g for 10 minutes at 4°C. Supernatants were collected and stored in 25
19 mM bicine buffer with 20% glycerol at -20°C.

20 We also tested the effects of various amounts of filter extracts for their ability to induce
21 luciferase activity in H1L1.c2 cells. In these experiments, cells were seeded at 150,000
22 cells/well and after growth overnight, cells were incubated with sample extracts for 4 h with
23 various amounts of DCM extracts, roughly equal to what an adult male might inhale in 1 to 515
24 minutes or 0.068 – 5.0 m³ air. Extract volumes used were 0.5% of the total incubation volume in

1 each well. Following exposures, cells were rinsed with calcium and magnesium-free phosphate-
2 buffered saline and lysed with 100 μ l of lysis buffer (Promega, Madison, WI). Supernatants
3 were collected and stored at -20°C.

4 **Biochemical Assays**

5 Ethoxyresorufin O-deethylase (EROD) activity was measured fluorometrically as
6 previously described (Sanderson et al. 1996). In a 96 well Microlite-1 plate (Dynex, Chantilly,
7 VA), 30 μ l of cell homogenates were mixed with 100 μ l of 50 mM HEPES buffer, pH 7.8,
8 containing 40 μ M dicumarol, and 50 μ l of a 20 μ M stock solution of ethoxyresorufin with 1.25%
9 Tween 80. Wells were monitored for background activity for 5 minutes prior to the addition of
10 50 μ l 0.5 mM of NADPH, used to start the reaction. Excitation/emission wavelengths of
11 535/585 nm respectively, were used to monitor EROD activity, which was expressed as
12 pmol/min/mg protein. Protein concentrations were determined using the bicinchoninic acid
13 (BCA) protein assay (Pierce Chemicals, Rockford, IL) with bovine serum albumin as the
14 standard.

15 Luciferase activity in 25 μ l H1L1.1c2 homogenates was determined in white opaque 96
16 well Cliniplates (Labsystems, Franklin, MA) followed by addition of 100 μ l luciferase assay
17 reagent (Promega, Madison, WI) as previously described (Garrison et al. 1996). Luciferase
18 activity was quantitated for 10 seconds and activity expressed as relative light units
19 (RLUs)/min/mg protein. The protein concentrations were also determined using the BCA assay
20 (Pierce Chemicals, Rockford, IL).

21 In order to identify whether any of the extracts had the potential to produce overt toxicity
22 in the cell lines, we used the XTT assay (Roehm et al. 1991). This assay measures the
23 mitochondrial metabolism of the tetrazolium dye 2,3 bis[2-Methoxy-4-nitro-5-sulfophenyl]-2H-
24 tetrazolium-5-carboxyanilide (Sigma, St. Louis, MO). Cells were exposed to various

1 concentrations of DCM extracts collected from the three PM₁₀ sites along with various
2 concentrations of DMSO (0.5, 1, 2%) and a negative control.

3 **In Vitro Bioassays**

4 Based on results of the range finding studies, H4IIE cells were exposed to an amount of
5 extract equivalent to 0.145 m³ air per well while H1L1.1c2 cells were exposed to an amount
6 equivalent to 0.0145 m³ air. Sample extracts for the month of December, January, and February
7 from the Northeast, Tillman, and Advance sites were used for cell exposure studies. Twenty-
8 four hours after sub-culturing in 6 well plates, DCM extracts were added to H4IIE cells for an
9 additional 24 h. Cells were then harvested, washed with Hanks buffer twice, and protein
10 extracted with 30µl of M-PER™. Cells were removed, centrifuged, and supernatant aliquots
11 were stored at -20°C. EROD activity measured as previously described. H1L1.1c2 cells were
12 cultured in 24 well plates overnight, seeded at a density of 150,000/well, then exposed to DCM
13 extracts for 4 h, cells washed and lysed as previously described, then immediately assayed for
14 luciferase activity. Cells used for positive and negative controls were exposed to either 1 µM β-
15 naphthoflavone or DMSO respectively. Both the EROD and luciferase assays were performed in
16 quadruplicate.

17 **Chemical Analysis**

18 Extracts from the Advance site were also analyzed for the concentration of selected
19 PAHs. A small aliquot of each of the DCM extracts was diluted ten fold (20 µl extract/200 µl
20 DCM) so the concentration of the target analyte fell within the linear range of the analytical
21 method (~ 0.05 – 50 ng/µl). Samples were then spiked with a co-injection of standard (~0.1
22 ng/µl) that consisted of deuterated PAHs (chrysene-d₁₂ – perylene-d₁₂) purchased from
23 AccuStandard, Inc. (Z-014J).

1 Samples were analyzed by the electron impact gas chromatography/mass spectrometry
2 (GC/MS) technique, using a Hewlett-Packard 5890 GC interfaced to a 5972 Mass Selective
3 Detector operated in scan mode. One microliter splitless injections were made onto a 30m DB-
4 5ms capillary column (0.25 mm i.d., 0.25 μ m film thickness) with an HP 18596A Autosampler.
5 Quantification of compounds was conducted by comparing the response of the analyte to the
6 response of the deuterated co-injection standard that most closely matches its retention time. To
7 correct for the varying response factors encountered for compounds with different molecular
8 structures, calibrations were created using authentic standards purchased from Aldrich Chemical
9 (Milwaukee, WI) and the National Institute of Standards and Technology (SRM 2260;
10 Gaithersburg, MD).

11 **Meteorological Data**

12 The morning mixing height data examined during the course of the study, processed from
13 the surface and upper air meteorological data at Santa Teresa, NM, was obtained from the
14 National Climatic Data Center, Ashville, NC. Santa Teresa, NM, located in the northwest corner
15 of the Paso del Norte airshed, provides the only available surface meteorological data for the
16 region. Mixing height measure the height above the ground level in which air freely mixes. The
17 correlation between mixing height and in vitro bioassays were made.

18 **Statistical Analysis**

19 Pearson's correlation coefficients were used to examine the relationship between
20 gravimetric analysis of PM₁₀ and the response variables. Simple regression analysis was used to
21 identify a relationship between morning mixing height and luciferase activity. Statistical analyses
22 were not performed on bioassay data since samples for each date and location came from a single
23 filter. Statistical Analysis System (SAS, Cary, NC) and Statview (Cary, NC) were used to
24 perform the analysis.

1 RESULTS

2 The gravimetric results were determined by weighing filters before and after PM₁₀
3 collection for filters obtained from the three sites on the 15 collection dates (data not shown). No
4 exceedances of the US EPA 24 hour standard of 150 µg/m³ of air occurred at Tillman or
5 Northeast, however 4 exceedances were observed at the Advance site. In general, PM₁₀ collected
6 at Advance was 6 – 8 fold greater than at Northeast and 2-3 fold greater than at Tillman.

7 Results of the range finding experiments are shown in Figure 1 (a & b). The DCM
8 extracts from Northeast and Advance that were equivalent to 0.03 – 0.17 m³ air caused an
9 increase in EROD activity. EROD activity in Tillman extracts declined over most of the
10 concentration range. Based on this study, H4IIE cells were exposed to extract equivalent to
11 0.146 m³ air for the remainder of the experiments. In contrast to the decline in activity observed
12 with EROD at higher amounts of extract, no such inhibition was seen with luciferase activity.

13 Cytotoxic effects were noted only when cells were exposed to extracts equivalent to 0.47
14 m³air from the Tillman and Advance sites, as indicated by the reduced metabolism of tetrazolium
15 dye in the XTT assay. We also noted increased vacuolization of H4IIE cells exposed to greater
16 than 5.0 m³ air collected from Tillman and Advance (data not shown). In contrast, no inhibition
17 or changes in cellular ultrastructure were observed with extracts from Northeast at any
18 concentration. We observed no cytotoxicity at extract doses used in either the EROD and
19 luciferase assays, which were 3 and 32 fold lower than the lowest cytotoxic dose.

20 The effect of exposures of H4IIE cells to extracts prepared from filters collected from the
21 3 monitoring sites over the 3 month period on EROD activity is shown in Figure 2. With one
22 exception (February 23), EROD activity in cells exposed to Advance extracts was highest of the
23 3 sites. EROD activity associated with the Northeast site was consistently the lowest of the three
24 for any single collection date. Typically, results obtained from Tillman samples fell in between
25 the two other values. On average, EROD activity from Tillman extracts was about 1.5 times

1 greater than Northeast and Advance extracts 1.5 – 2 times greater than Tillman. Only extracts
2 from Advance approached the activity of 11 pmol/min/mg protein, seen with the positive control,
3 1 μ M BNF. We have shown that this concentration of BNF produces a similar degree of AhR
4 induction as 10 nM TCDD (Garrison et al. 1996). Analysis of activity from Advance samples on
5 2 of the 5 days in January had similar levels of activity as cells treated with 1 μ M BNF (Figure
6 2b). EROD activity measured in Northeast POM obtained from the PM₁₀ filters was the most
7 consistent over time while those from Advance were the most variable.

8 The effects of exposures of extracts to H1L1.1.c2 cells containing the inducible luciferase
9 reporter system are shown in Figure 3. A similar pattern of induction was observed in these
10 experiments as was obtained in the EROD assay. Luciferase activity at Tillman was 1.5 times
11 greater than at Northeast; activity seen with Advance extracts being about 1.5 times those from
12 Tillman. Again, the exception to this rule was noted with the February 23 sample where Tillman
13 was highest and the difference between Advance and Northeast was only 50%. The fluctuations
14 in activity at Advance seen with the EROD assay were not as great with the luciferase assay.

15 There was a significant relationship between PM₁₀ mass (μ g/m³ in 24 hour) and both
16 EROD and luciferase activities (Figure 4). When data from all three sites were pooled, we
17 observed a positive correlation between PM₁₀ mass and the two major endpoints ($p < 0.0001$).
18 However, when this relationship was analyzed by site, the relationship was not as strong,
19 primarily because of clustering of data at the lower or higher end of the PM₁₀ mass range. We
20 found a statistically significant relationship between luciferase activity and filter weight at the
21 Advance and Tillman, but not at Northeast (data not shown).

22 To gain some insight into the role winter inversion layers might have on PM₁₀ and
23 associated POM, the relationship between morning mixing height and luciferase activity was
24 examined (Figure 5). During the winter, morning mixing height was very low, averaging less

1 than 100 meters most of the time. As a comparison, morning mixing heights of 250 – 350 meters
2 are common during the summer months. Different relationships between mixing height and
3 activity were found at the 3 sites. The strongest relationship was observed at Tillman ($p <$
4 0.006), a strong association was seen at Northeast ($p < 0.08$), and no relationship was noted at
5 Advance.

6 Lastly, the concentration of the target PAH analytes for 9 collection dates were measured
7 in POM collected from the Advance filters (Table 1). Sample dates are ordered according to the
8 PM_{10} collection on the respective date. In general, the total measured PAH concentration
9 increased with PM_{10} mass. PAHs detected in the highest concentrations were benzo[a]pyrene
10 (BaP), chrysene/triphenylene, benzo[b+j+k]fluoranthene, and benzo[ghi]perylene. One sulfur
11 containing and three oxygenated PAH compounds were also analyzed. The molecular weight of
12 the compounds investigated ranged from 234 for retene (1-methyl-7-isopropyl-phenanthrene) to
13 300 for coronene. Total PAH concentration was also reported for each date. We found a
14 positive correlation ($R^2 = 0.53$, $p < 0.03$) between PAH concentration and luciferase activity
15 (data not shown). On average, [PAH] was 12.7 ng/m^3 . As a reference, data from PM_{10} extracts
16 collected in 3 other cities in the Americas are provided as well in Table 1.

17 DISCUSSION

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

1 As expected, activities of both EROD and luciferase were greatest at the Advance site,
2 the PM₁₀ station adjacent to a brickmaking district in Juarez (Figures 2 and 3). In January, the
3 month in which the highest activity was recorded, luciferase activity was 1.7 times that measured
4 at Tillman and 2.5 times greater than Northeast. EROD activity at Northeast was consistently
5 low, similar to control activity of non-exposed H4IIE cells (0.85 pmol/min/mg protein).
6 However, luciferase was higher than control activity (58 RLU/mg protein), suggesting that small
7 amounts of AhR ligands were present in Northeast extracts. EROD and luciferase activity
8 associated with Tillman filters generally fell in between the response observed at the other two
9 sites. The average luciferase activity associated with DCM extracts from Northeast was 432; at
10 Tillman, 663; and at Advance, 994 RLU/mg protein. This level of induction was caused by
11 exposure to extracts equivalent to 0.145 m³ of air and represents 24%, 37%, and 55%
12 respectively of the activity seen with 1 μM BNF. These comparisons suggest significant
13 amounts of AhR ligands are present in POM, especially at the Advance site.

14 Both luciferase and EROD activities mirrored total PM₁₀ mass, as reflected by the
15 significant positive correlation between these variables (Figure 4). This relationship suggests
16 that AhR ligands contribute a constant percentage of the total POM associated with PM₁₀. In a
17 location such as the Paso del Norte airshed, we speculated that the contribution of silica-based
18 particles in PM might vary due to the desert terrain and high percentage of unpaved roads.
19 However, our findings suggest that this is probably not the case, at least not in the winter months.
20 It would appear that as total PM₁₀ increases, there is a concomitant increase in PAHs and other
21 AhR ligands.

22 To gain a better understanding of the potential for deleterious effects of the POM that
23 induced AhR mediated activity measured in this study, we identified work of other investigators
24 who analyzed PAHs in particulate matter extracts from a number of cities. Massolo et al., (2002)
25 assessed the chemical composition, mutagenicity, and toxicity of particulates from La Plata,

1 Argentina and Leipzig, Germany. Extracts from industrial areas in La Plata contained
2 approximately 2.0 ng Benzo[a]pyrene (BaP)/m³ air collected via a high volume sampler
3 (Massolo et al. 2002). Benzo[a]pyrene in our samples collected from Advance ranged from a
4 0.19 to 4.48 ng BaP/m³, with a geometric mean of 1.3. Extracts from the winter La Plata
5 samples were used for mutagenicity testing using the TA 98 strain with an S9 microsomal
6 fraction (TA98 + S9); the results showed an average of 2.5 revertants/m³ compared to 0.16
7 revertants/m³ at the reference site. These investigators found a significant correlation between
8 the levels of carcinogenic PAHs and BaP and mutagenicity. They also found that the highest
9 mutagenicity was associated with particles 1.5 µm or less, those that have the highest likelihood
10 of reaching the alveoli and deep respiratory tract. In a study in La Spezia, Italy; Barale et al.
11 (1991) found BaP concentrations of 1.6 ng/m³ at one of their study sites, a concentration
12 comparable to our measurements at Advance (Barale et al. 1991). Although mutagenicity of
13 samples with this level of BaP was not identified in their report, they found that approximately
14 10 ng/m³ total PAHs (15 were measured), the number of revertants averaged 2.5/m³ (TA98 +
15 S9). Gil et al. (2000) found a much higher level of mutagenicity in their study in Santiago,
16 Chile. Using organic extracts from PM₁₀ filters, they measured between 200 – 600 revertants/m³
17 (TA 98 + S9) in samples with a concentration of about 5.0 ng BaP/m³ (Gil et al. 2000). These
18 studies provide some frame of reference regarding the potential mutagenic effects that might be
19 associated with the AhR-linked activities and PAH concentrations that were measured at the
20 Advance site in this study.

21 PAHs identified in this study include some that are listed by the US EPA as hazardous air
22 pollutants because they have the potential to cause adverse health effects, including cancer,
23 cardiopulmonary disease, and developmental and reproductive toxicity. The results of our
24 studies suggest that neighborhoods located near industrial sites may be at increased risk for these

1 environmentally-linked conditions. One method of estimating the public health risk associated
2 with exposure to PAHs is to calculate the risk of cancer. To do this, we used the US EPA oral
3 slope factor of 7.3×10^0 (mg/kg)/day and adjusted this value for the weight of a 70 kg male, and
4 an inhalation rate of $20 \text{ m}^3/\text{day}$ in the following calculation: $(7.3 \times 70) / 20 = 25.55 \text{ (mg/m}^3\text{)/day}$
5 or 2.56×10^{-5} (ng/m³)/day (U.S. EPA 1987). The concentrations of the 7 major PAHs measured
6 at the Advance site were converted using toxic equivalency factors based on US EPA guidance
7 (1993) to obtain benzo[a]pyrene (BaP) equivalents (U.S. EPA 1993). This value averaged 1.97
8 ng BaP equivalents/m³ over the sampling period. Multiplying the BaP equivalents by the slope
9 factor yielded the value of 5.03×10^{-5} or 5 cancers per 100,000 people. This value represents an
10 estimate of the number of cancers associated with a lifetime exposure to PAHs at the average
11 concentration measured during the winter at Advance. Some of the limitations of this estimate
12 include the fact that data for the slope factor was derived from oral exposures and that personal
13 exposure is not necessarily at the same concentrations as those collected by the PM₁₀ samplers.
14 For comparative purposes, we used the World Health Organization (WHO) Air Quality
15 Guidelines for Europe's unit risk factor of 9×10^{-5} per ng BaP/m³ to make a second risk
16 calculation from ambient PAHs; an estimate of 1.2 cancer cases per 10,000 was obtained
17 (Borstrom et al. 2002). Although the factors used in the US EPA and WHO calculations were
18 based on different types of data, the results suggest a comparable risks: between 5 and 12 cases
19 per 100,000 people.

20 Limitations of this analysis include the fact that risk estimates are based on PAH
21 concentrations measured in winter, when the combination of inversion layers, higher emissions
22 from combustion sources, and reduced atmospheric degradation all lead to higher concentrations
23 than would likely be found in other months of the year. Nonetheless, these estimates suggest a
24 considerable risk associated with exposure to PAHs, especially in light of the fact that the US
25 EPA considers a cancer risk of between 1 in 100,000 - 1,000,000 as not significant; the State of

1 California's Proposition 65 program uses 1 in 100,000 as the no significant risk level. This
2 estimate can be compared to an estimate of cancer risk associated with urban life in
3 industrialized nations (Hemminki and Pershagen 1994). These workers calculated a risk of 1
4 cancer case per million people per year among urban residents exposed to an average ambient air
5 concentration of 0.7 ng BaP/m³. Ambient concentrations of PAHs at other sites in the airshed as
6 well as the concentration of other important toxic air pollutants, such as nitro-PAHs, PCBs, and
7 dioxins, are not known and should be investigated in the future to obtain a more accurate
8 estimate of risk.

9 In addition to cancer risk, developmental and reproductive toxicity are associated with
10 PAH exposure. Three-day-old chick embryos exposed to crude extracts of urban air from
11 Teplice, Czech Republic containing carcinogenic PAH mixtures at a concentration of 49.5
12 ng/m³, developed defects in the heart and abdominal wall (Binkova et al. 1999). Carcinogenic
13 PAHs such as BaP were present at levels of 7.42 ng/m³ during the winter in Teplice.
14 Furthermore, carcinogenic PAHs at concentrations greater than 15 ng/m³ have been known to
15 attenuate fetal growth in humans (Dejmek et al. 1999). At Advance the highest concentration of
16 BaP was about one-half of this value, 4.88 ng/m³ during the winter. The concentration of total
17 PAHs was on average 12.8 ng/m³, similar to that seen in the Dejmek et al. study. In this study,
18 WBC DNA-adduct levels were higher in infants (7.9 ± 0.93 per 10^8 nucleotides) than their
19 mothers (5.9 ± 0.77 per 10^8 nucleotides). These findings suggest that infants might be at greater
20 risk of DNA damage because of their reduced ability to detoxify these types of contaminants
21 (Perera et al. 1999). The presence of these contaminants in the Paso del Norte airshed would
22 suggest there is a risk for developmental toxicity that needs further investigation.

23 The chemical analysis of PM₁₀ extracts collected from Advance showed that the
24 concentration of most PAHs were higher than the average ambient concentration reported for

1 Toronto (Clemons et al. 1998) and in some cases for Denver (Chow 1998). However, PAH
2 concentrations at Advance were consistently lower than in Santiago (Gil et al. 2000). The upper
3 limit of the concentrations in Toronto and Denver are comparable to the highest concentrations at
4 the Advance site for benz[a]anthracene, retene, and coronene. For many of the other
5 compounds, however the higher concentrations at Advance are approximately 2 – 3 times higher
6 than the three cities identified above. For example, the concentrations of
7 benzo[g+j+k]flouranthene and benzo[ghi]perylene on certain dates was approximately double
8 than the highest value reported in Denver. Consistent with other assessments of ambient air
9 quality, the PAHs quantified here probably account for a small percentage of POM (Seinfeld and
10 Pandis 1998).

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

21 The interaction between emission sources and climatic conditions needs to be considered
22 when evaluating the potential health effects of urban air pollutants (Moller et al. 1994). In
23 particular, an understanding of the influence of meteorological conditions could assist in
24 estimating possible impact of inversion layers on the distribution and concentration of POM
25 within the airshed. The most dominant weather characteristic in the winter in the Paso del Norte

1 airshed are the presence of inversion layers during winter months, which regularly reduce mixing
2 height to less than 100 meters. In the present study, different relationships between morning
3 mixing height and luciferase activity were seen at the 3 sites. At Advance, no relationship was
4 observed, suggesting that localized emissions, most likely associated with brickmaking activity,
5 were the main influence on the content of the POM. Regardless of the presence of an inversion
6 layer, luciferase activity was consistently high. This contrasts with Tillman, where a positive
7 correlation existed between the morning mixing height and luciferase activity. The Tillman site
8 is located in downtown El Paso near the center of the basin that forms the Paso del Norte airshed.
9 It is within blocks of the Sun Metro main bus depot and a bridge, a major crossing between El
10 Paso and Ciudad Juárez that has heavy truck and automobile traffic. Due to its location, one
11 would anticipate that the presence of an inversion layer would significantly affect POM at
12 Tillman.

13 In contrast to Tillman, this relationship was weaker at the Northeast site ($p < 0.08$).
14 Located near the edge of El Paso, normally PM_{10} is lowest in this area of the city. However,
15 when inversion layers occur, it is apparent that PM_{10} generated in other areas disperses
16 throughout the airshed and changes the character of POM at this site. What is not clear is the
17 degree to which either a) particles generated by traffic and/or brickmaking activity many miles
18 away in Juárez influences the conditions at sites in El Paso or b) the degree to which mobile
19 source combustion by-products generated in El Paso affect POM in Ciudad Juárez during an
20 inversion layer. The shallow winter inversion layer coupled with complex terrain topology,
21 potential nighttime drainage flow, native vegetation detritus, vehicle-enhanced road dust, and
22 increased emissions of biomass combustion and waste burning in the wintertime Paso del Norte
23 complicate the relationship between PM_{10} and AhR ligands (Li 1999; Li et al. 2001). Combining
24 data generated by the present study with topographical and air dispersion models in a
25 geographical information system (GIS) would be needed to answer this question. One example

1 of this approach was used by researchers in Stockholm (Bellander et al. 2001) who used GIS to
2 assess historic exposure to NO₂ and SO₂ in various neighborhoods throughout the city.
3 Geographically coding the PM₁₀ sampler sites throughout the airshed and including AhR
4 mediated activity in a GIS analysis would provide useful information regarding potential
5 exposure of people throughout the airshed to harmful contaminants.

6 **CONCLUSION**

7 This work represents a first attempt to identify toxic chemicals associated with PM₁₀ in
8 the Paso del Norte airshed. Of the two bioassays used to detect AhR ligands, the luciferase
9 reporter assay was the more sensitive. Our approach of using in vitro assays such as these to
10 identify potential sources of POM provides rapid results and conclusions that are complementary
11 and easier to achieve than the traditional, labor-intensive, source apportionment and principal
12 component analysis. In addition, these cell bioassays can be used for screening large numbers of
13 sample extracts to identify those for subsequent expensive instrumental analysis.

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

20

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TABLE 1: Comparison of Polycyclic Aromatic Hydrocarbons (PAHs) values in the Paso del Norte (Advance Site) PM₁₀ Air Samples Compared to Literature Values of Other Cities.

Date	Santiago 1996 ^a	Denver, 1997 ^b	Toronto, 1998 ^c	ADVANCE SITE PAHs (ng/m ³)									
				12/1/98	12/7/98	12/19/98	12/31/98	1/6/99	1/18/99	1/24/99	2/11/99	2/17/99	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	.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	.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	.40	3.0	1.5	7.9	5.3	7.4	3.0	4.9	0.5	2.2	3.0
Benzof[e]pyrene		0.1-1.6	.18	1.2	0.5	2.7	2.4	3.3	1.3	1.7	0.2	0.8	1.2
Benzof[a]pyrene	4.9	0.1-1.5	.06	1.0	0.5	3.9	2.9	4.5	1.8	2.3	0.2	0.5	1.3
Perylene			.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		.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	.25	1.3	0.4	2.4	0.2	3.1	1.5	1.9	0.2	0.7	0.9
Benzof[ghi]perylene	7.6	0.1-2.8	.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 PAH													
Benzonaphthothiophene				0.2	.04	ND	0.3	0.3	ND	0.4	ND	0.5	0.3
Oxygenated PAH													
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-chrysoquinone				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

^aGil et al., 2000, ^bChow et al., 1998 ^cClemons et al., 1998 ; ND = Not detectable
GM = the geometric mean.

Legend

Figure 1a and b. Effects of varying amounts of PM extracts on bioassays.

1a: Effects of PM extracts from Dec. 7, 1998 filters on EROD activity. Bars represent mean \pm SEM of 4 replicates. H4IIE cells were exposed to extracts from particles collected from 0.03 – 9.92 m³ filtered air for 24 hours.

1b: Effects of PM extracts from Dec. 7, 1998 on luciferase activity. Bars represent mean \pm SEM of 4 replicates. H1L.1c2 cells containing an AhR driven luciferase reporter system were exposed to extracts containing between 0.01 – 5.01 m³ filtered air for 4 hours.

Figure 2. Effects of PM₁₀ extracts on EROD activity. H4IIE cells were exposed to extracts equivalent to 0.145 m³ air from the three PM₁₀ monitoring sites. Data points represent the mean \pm SEM of 4 replicates. Panel A shows EROD activity associated with December, 1998 filters; Panel B shows data from January, 1999; Panel C contains data from February, 1999 filter collections. Negative control activity was 1.14 pmol/min/mg protein; positive control activity with 1 μ M BNF was 11 pmol/min/mg protein.

Figure 3. Effects of PM₁₀ extracts on luciferase activity. H1L.1c2 cells were exposed to 0.0145 m³ air from the three PM₁₀ monitoring sites. Data points represent the mean \pm SEM of 4 replicates. Panels contain data as described for Figure 4. Negative control activity was 18 RLU/mg protein; positive control with 1 μ M BNF was 1810 RLU/mg protein.

Figure 4. Relationships between PM₁₀ gravimetric weight and bioassay activities. Each data point represents the mean of 4 replicates. EROD units are pmol/min/mg protein. Luciferase units are RLU $\times 10^{-2}$ /mg protein. Superscripts indicate significant difference from zero at $p < 0.001$ level. r values are shown for each regression line.

Figure 5. Relationships between morning mixing height and luciferase activity. Data points represent the mean of 4 replicates. Mixing height data was obtained from the U.S. National Weather Service.

Table 1.: Comparison of Polycyclic Aromatic Hydrocarbons (PAHs) values in the Paso del Norte (Advance Site) PM₁₀ Air Samples Compared to Literature Values of Other Cities. Data represents analysis of a single aliquot of dichloromethane extract analyzed by GC/MS from filters collected between December 1998-February 1999. The values for all compounds are reported as ng/m³. For comparative purposes, data from three other metropolitan areas are shown. GM = the geometric mean.

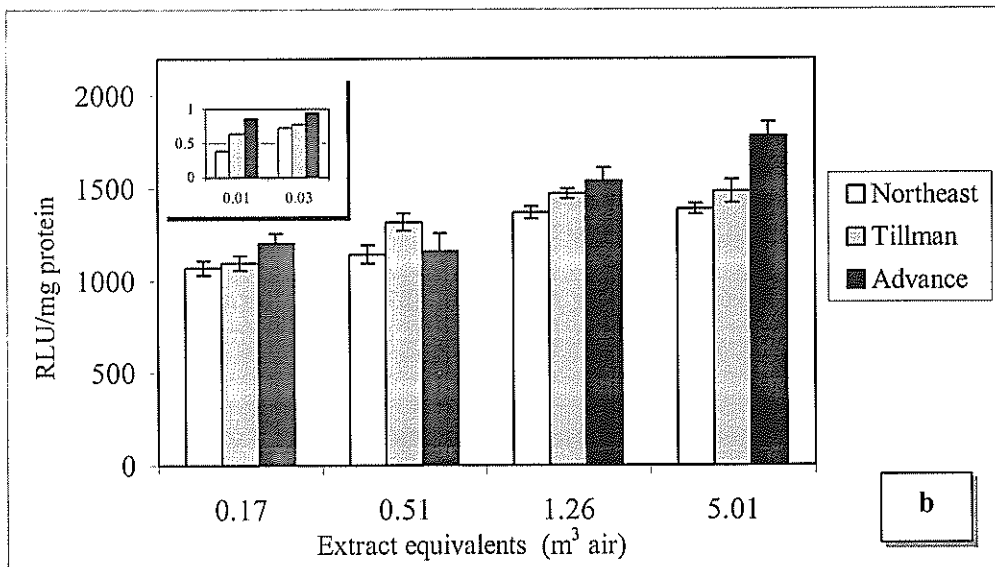
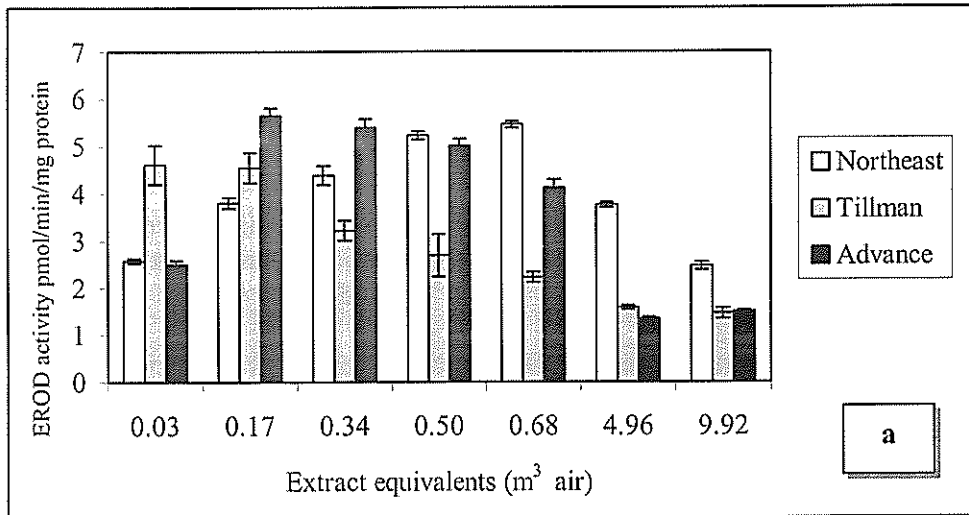


Figure 1.

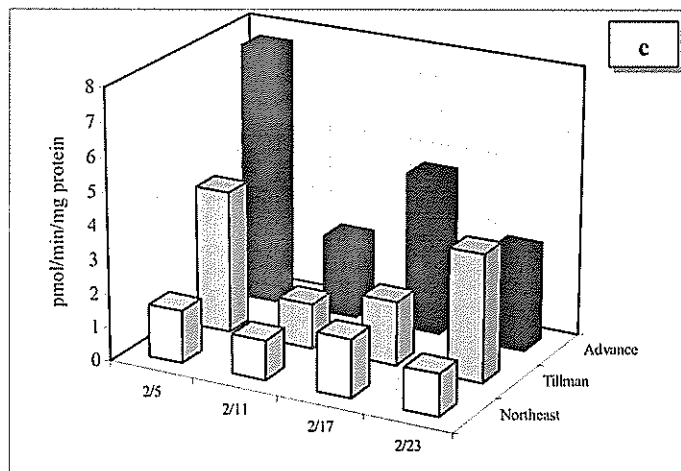
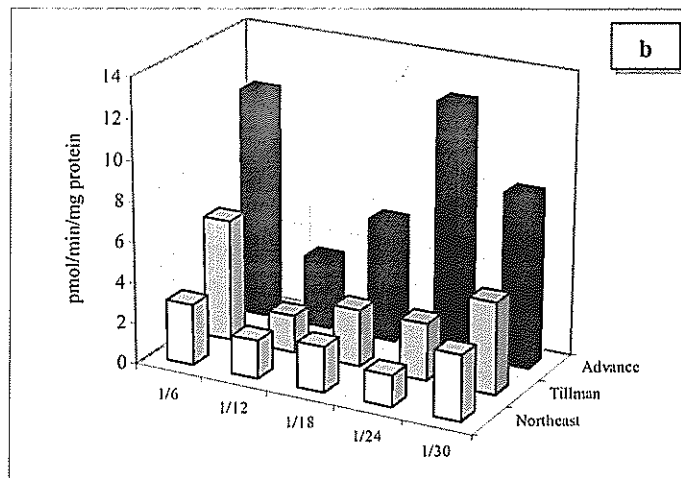
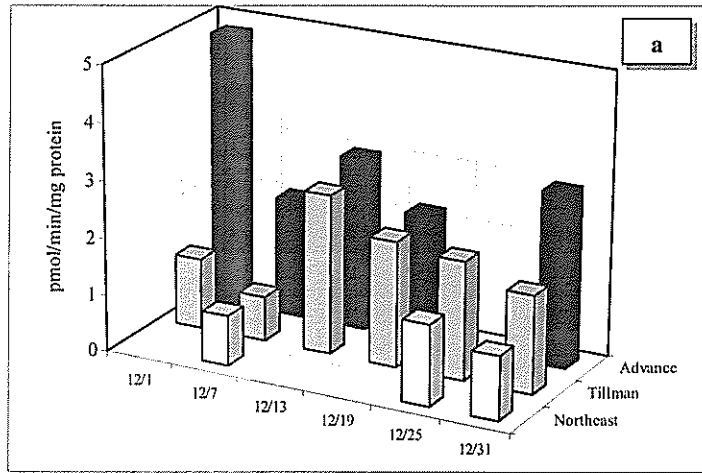


Figure 2.

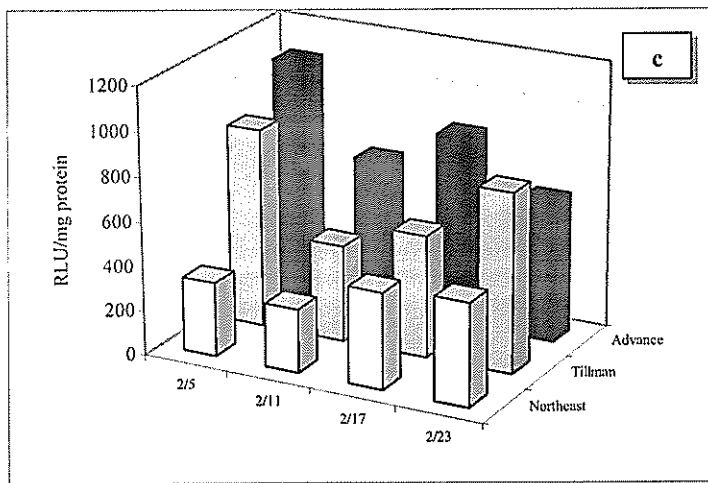
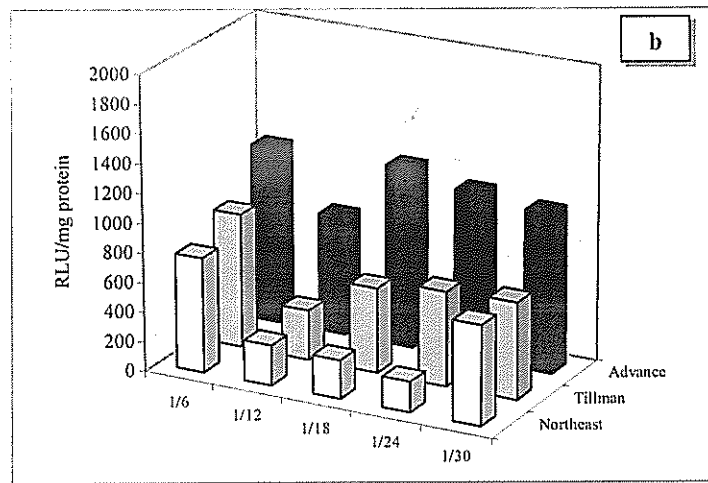
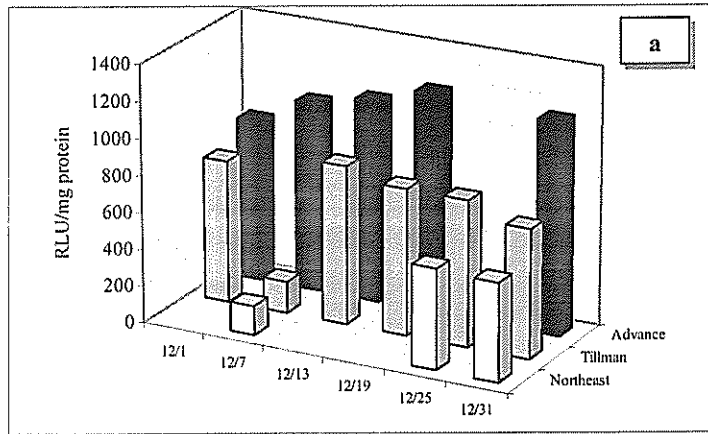


Figure 3.

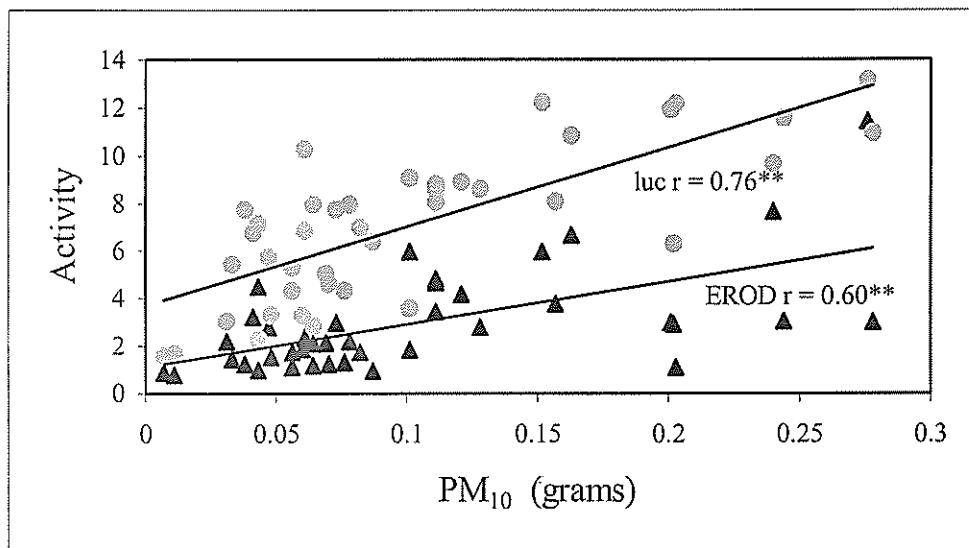


Figure 4.

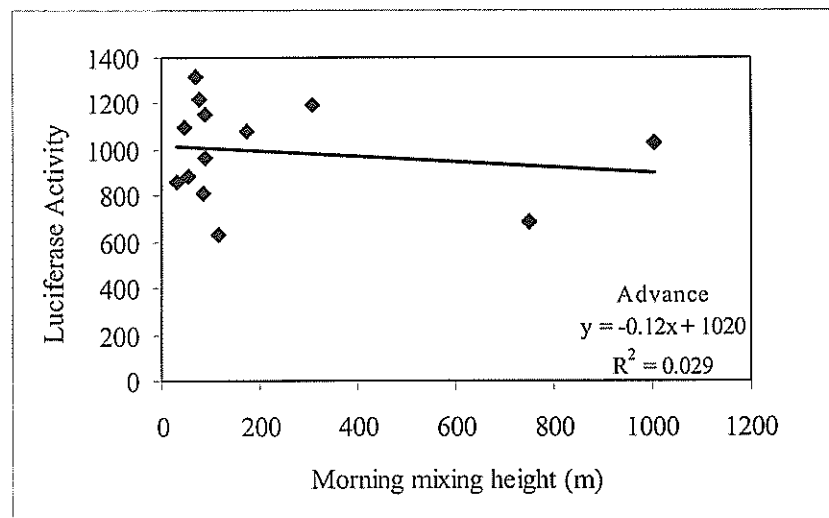
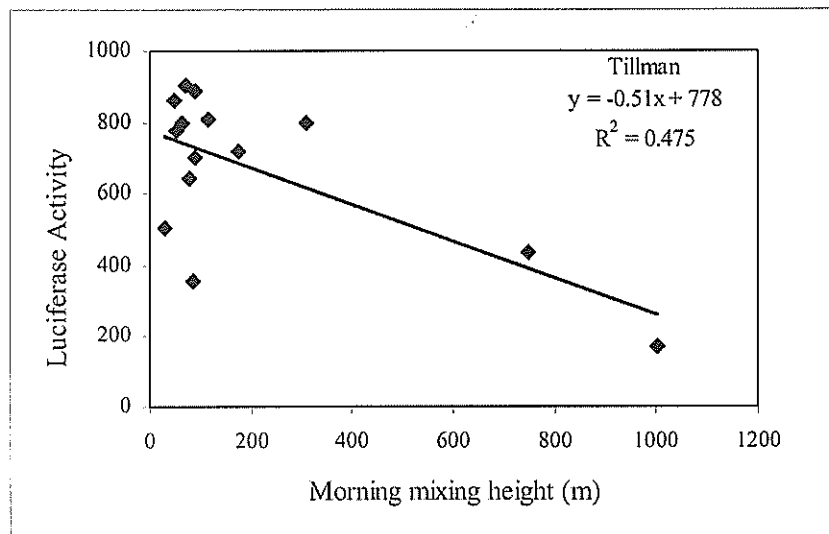
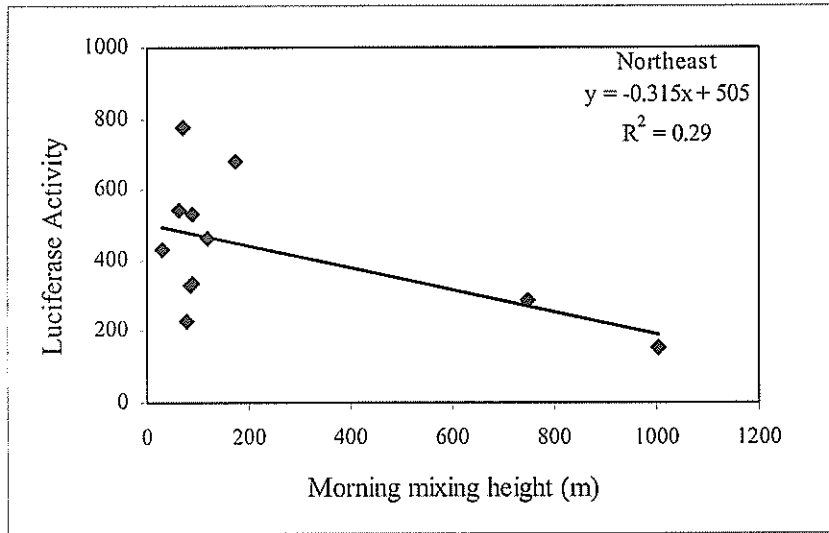


Figure 5.