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HOUSTON AIR TOXICS BIOMARKERS OF EXPOSURE STUDY (HATBES)

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Abstract

Industrial activities and the consumption of fossil fuels release a broad range of organic and inorganic chemicals into the environment. Although these chemicals may come into contact with air, water, soil or sediment, air is usually considered one of the primary pathways of exposure. Air samples can be collected to estimate population exposures; however, minimal data exists to relate air exposures to biomarkers in serum or urine. The goal of the Houston Air Toxics Biomarker of Exposure Study (HATBES) was to investigate the utility of measuring various biomarkers of exposure to volatile organic compounds (VOCs) in human populations. The original intent of this study was to integrate with two on-going studies in Houston: one which is monitoring air quality and personal exposure (the Houston Exposure to Air Toxics Study or HEATS) and one which is examining biomarkers of inflammation (a University of Texas-Medical Branch study). However, due to unforeseen circumstances, which included difficulties with recruiting and retaining volunteers after Hurricane Ike, HATBES received permission from the Texas Commission on Environmental Quality (TECQ) to recruit from the general population from two areas in Houston, Texas. The original goal of the study was to recruit 15-20 participants from each area to evaluate sensitive biomarkers of exposure to VOCs. Then, if an appropriate analytical measurement was identified, Phase II would recruit a larger population and continue measurements in the initial population. However, after the focus changed due to recruitment issues, the goal of this study was to recruit 50 adult participants each from the Manchester (Study Population) and Aldine (Reference Population) neighborhoods for biomarker evaluation. Biological samples were collected from participants on a single sampling date for each community rather than in two phases as originally proposed. A brief activity questionnaire was administered prior to sample collection in order to provide information on confounding sources of exposure (i.e. hobbies, occupation, lifestyle, etc.). A maximum of 20 mL of serum and 40 mL of urine were collected from each adult. Whole blood and urine were extracted and analyzed for biomarkers of exposure to benzene, toluene, ethylbenzene and the xylene isomers (BTEX), and 1,3-butadiene. The TCEQ provided every 6th-day 24-hour ambient air sampling data from TCEQ monitors located in the Aldine and Manchester areas. These data were used to correlate chemical concentrations in air to chemical concentrations in blood and urine in the two communities. A critical component of this project was support from the community. Suggestions of interventions that may reduce exposure and risk will be provided to the community at the conclusion of the project. A long-term goal of the study is to hopefully prevent disease by reducing exposures.

Introduction

Background

Complex chemical mixtures are prevalent in media such as air, water and soil. Industrial activities and the consumption of fossil fuels from petrochemical industries release a broad range of organic and inorganic chemicals into the environment. The potential for these exposures to cause adverse health effects in humans depends on the dose and duration of exposure, the toxicity of specific chemicals, and a variety of individual factors such as genetics, nutrition and lifestyle. Chemicals present in the air are of particular interest for communities living adjacent to petrochemical facilities and in high vehicle traffic areas. Exposure to air toxics in residential settings are difficult to assess because levels are generally low and exposures may be intermittent (Lin et al. 2008). This poses a problem for understanding and managing health risks associated with exposure to ambient pollutants.

Volatile Organic Compounds (VOCs) are some of the most common contaminants detected in urban air (Caro et al. 2009). The United States Environmental Protection Agency (EPA) defines VOCs as "any organic compound that participates in atmospheric photochemical reactions except those designated by EPA as having negligible photochemical reactivity" (EPA 2006). Exposure to VOCs can cause multiple adverse health effects such as neurological, genotoxic and developmental effects (Senzolo et al. 2001). Many VOCs have been identified as carcinogens. Two VOCs of particular concern in Houston, Texas are benzene and 1,3-butadiene (Whitworth 2008). The International Agency for Research on Cancer (IARC) recognizes benzene and 1,3-butadiene as known human carcinogens based on studies that showed sufficient evidence of increaed leukemia's in humans (IARC 1987, Grosse et al. 2007). The EPA classifies benzene as a known human carcinogen by all routes of exposure (EPA (a) 1998) and 1,3-butadiene as carcinogenic to humans by inhalation exposure (EPA (b) 1999).

The EPA Toxic Release Inventory (TRI) for 2005 reports the state of Texas released over one million tons of VOCs from the top five source sectors inluding: industrial processes, on road vehicles, solvent use, non road equipment, and other miscelleous sources. Total emissions nationally in 2005 for the same source sectors released fourteen million tons of VOCs (EPA (d) 2009). According to the EPA TRI, over 1.6 million pounds of hazardous air pollutant (HAP) chemicals were released from industrial facilities in Harris County in 2008. Of these 123 HAP chemicals, 1,3-butadiene, benzene, toluene, ethylbenzene and xylene isomers accounted for over one-third of the total HAP releases and were ranked in the top 11 of total on and off-site chemical releases (EPA (e) 2009). Although, releases of the carcinogens benzene and 1,3-butadiene in Harris County have decreased dramatically over the past decade, these chemicals still accounted for over one million pounds total on- and off-site releases in 2008 (Figure 1) (EPA (e) 2009).

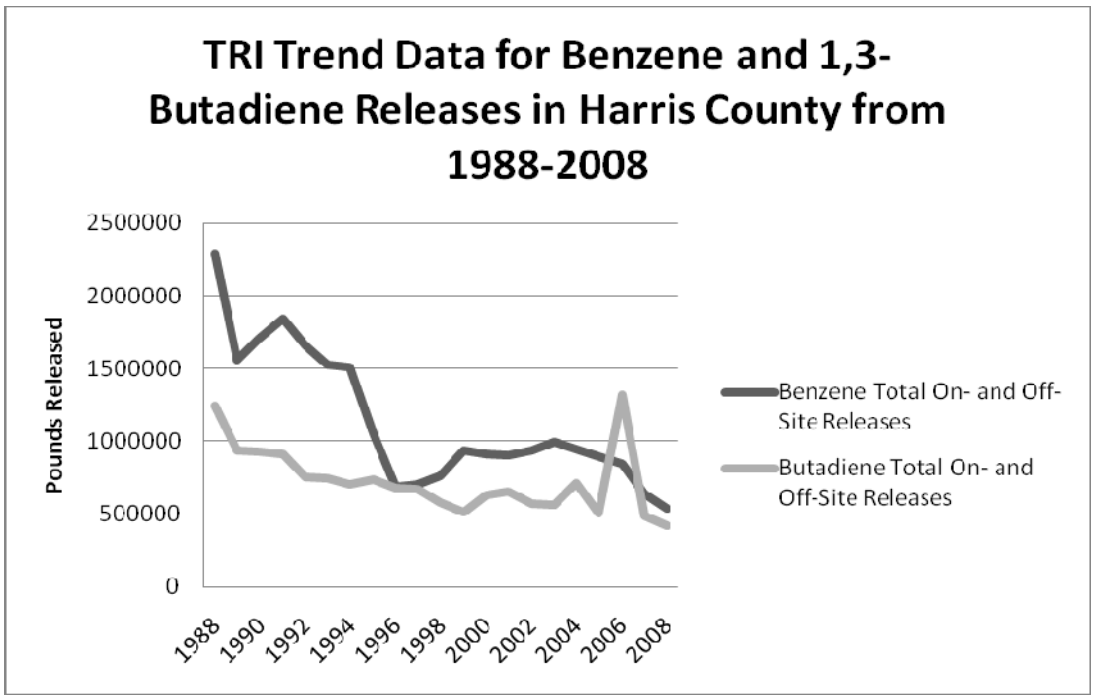

Figure 1. TRI on-site and off-site reported disposed of or otherwise released (in pounds), trend report for facilities in all industries, benzene and 1,3-butadiene, Harris County, Texas, 1988-2008.

Humans may be exposed to VOCs at the workplace, at home and while outdoors. A number of factors influence the dose and duration of VOC exposures in humans, including: time spent indoors and outdoors, activity and breathing levels, occupation, and hobbies or other activities, such as smoking status, that may result in increased exposures. When industrial activities release VOCs into the environment, these chemicals come into contact with different environmental media such as air, soil and water (Lin et al. 2008). The physical properties of VOCs make it easy for them to partition into the air; therefore, inhalation is considered the primary route of exposure to VOCs (EPA (a) 1998). Although air monitoring data provides important information regarding chemical concentrations in the environment, it is difficult to link this information to actual personal exposure and then to the internal dose taken up by individuals.

Over the past several years, human biomarker studies have been conducted to measure parent VOC compounds in whole blood and urine using solid phase micro-extraction gas chromatography/mass spectrometry (SPME GC/MS) (Manini et al. 2006, 2008; Lin et al. 2008, Hoet et al. 2008). This method is more sensitive and accurate than prior methods of VOC analysis as it measures parent VOC compounds in serum and urine without prior extraction procedures. Other studies have been conducted to look at parent VOC compounds in blood and urine, but the methods (ie. purge and trap) for analysis are less sensitive (Ashley et al. 1994, Sexton et al. 2005 and 2006). A biomarker study by Hoet et al. (2008) measured blood benzene levels in 110 male petrochemical workers and found a median concentration of 0.375 and 0.510 ng/ml in non-smokers and smokers, respectively. This study measured the lowest concentration of blood benzene levels in an occupational setting reported in literature to date utilizing the SPME GC/MS method. Lin et al. (2008) published one of the first studies to measure VOCs in a

non-occupational and unexposed residential population using the SPME GC/MS method. Blood was obtained from 354 participants in the 1999-2000 National Health and Nutrition Examination Survey (NHANES). Within this group representing the US general population, results showed the weighted geometric mean for blood benzene levels in non-smokers and smokers was 0.084 and 0.288 ng/ml, respectively.

The ^{32}P-postlabeling assay for deoxyribonucleic acid (DNA) adducts is an ultrasensitive method developed in the early 1980's for the detection and quantitation of carcinogen-DNA adducts. Measuring DNA adducts has a wide range of applications in human studies and can be used to detect events resulting from environmental exposures, adducts formed by environmental carcinogens and complex environmental mixtures (Phillips et al. 2007). DNA adduct levels have been used to assess exposures to carcinogens such as polycyclic aromatic hydrocarbons (PAHs). These adducts are referred to as bulky DNA adducts (Naufal et al. 2009). Some oxidative DNA damage that is induced by contaminants can also be determined by the postlabeling assay (Randerath et al. 2001, Godschalk et al. 2003, Zhou et al. 2004). In particular, type II I-compounds represent useful biomarkers of oxidative DNA damage such as 8,5'-cyclo-2'-deoxynucleotides. These bulky DNA oxidative lesions are induced by reactive oxygen species (ROS) which play important roles in mutagenesis, carcinogenesis and aging. Major type II I-compounds that have been identified represent base-base or base-sugar intrastrand crosslinks, and have been linked to ROS by *in vitro* and *in vivo* experiments which include the exposure to pro-oxidant chemicals. These type II I-compounds contain an 8,5'-cyclo-2'-deoxyadenosine (cA) oxidative lesion that has biological properties of a bulky DNA adduct. Randerath et al. 2001 found four I-compounds that are dinucleotides containing the cA adduct (Figure 2): pAp-cAp (AcA), pCp-cAp (CcA), pGp-cAp (GcA) and pTp-cAp (TcA) (Randerath et al. 2001). Other factors that may initiate ROS include ionizing radiation, exposure to foreign compounds (i.e. asbestos exposure), transition metals (i.e. iron, copper and nickel), and certain indirectly acting carcinogens (i.e. peroxisome proliferators) (Randerath et al. 1991). Overall, type II I-compounds are excellent biomarkers of oxidative DNA damage and can be easily detected by the P1-enhanced version of the ^{32}P-postlabeling assay (Randerath et al. 2001, Zhou et al. 2004).

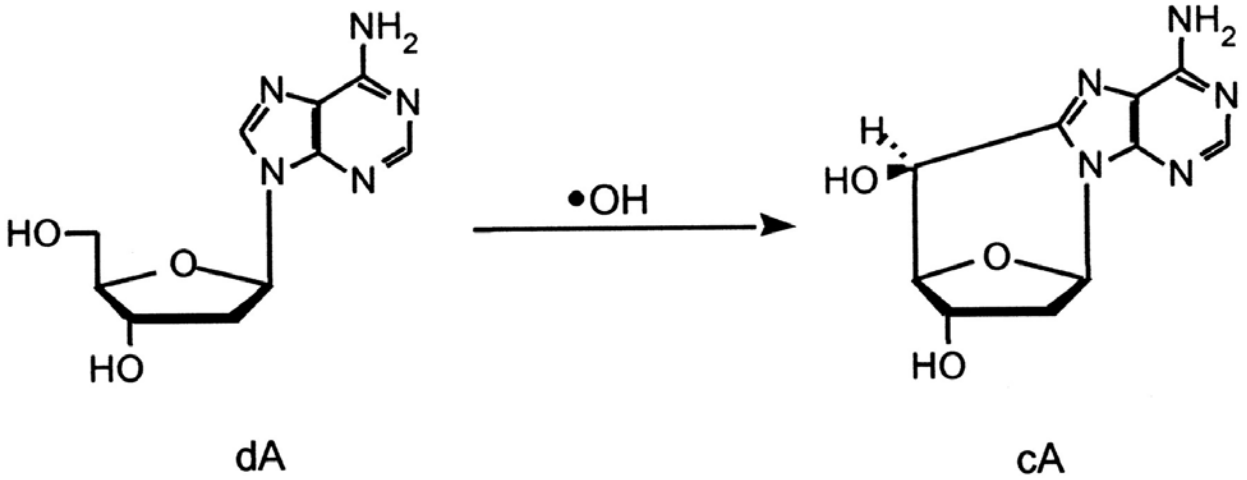

Figure 2. Formation and structure of cA. Hydroxyl radical induces formation of cA from 2-deoxyadenosine (*dA*) (Randerath et al. 2001).

Specific Aims

This study measured specific VOC biomarkers in biological specimens. Biomarkers provide valuable data regarding the exposure to specific compounds (Groopman et al. 2005). Traditional biological methods are not sensitive enough to detect VOC exposures resulting from low concentrations of chemical exposure. Therefore, there is a need to identify methods that are able to detect exposure to low levels of chemicals. The specific aim of this study was to investigate the utility and sensitivity of measuring various biomarkers of exposure to VOCs in human populations. The original intent of this study was to integrate with two on-going studies in Houston: one which is monitoring air quality and personal exposure (HEATS) and one which is examining biomarkers of inflammation (Dr. Jonathan Ward's study with UTMB). HEATS would have provided information on individual levels of exposure, while the biomarker of inflammation research would provide information regarding possible biomarkers of effect. However, due to unforeseen circumstances, which included difficulties with recruiting and retaining volunteers after Hurricane Ike, HATBES received permission from TECQ to recruit from the general population. Although the collaboration with HEATS was not possible, the collaboration with UTMB was successful. Texas A&M University (TAMU) and UTMB participated in all study recruitment and sample collections together and shared all participant data.

This study focused specifically on BTEX and 1,3-butadiene as the chemicals of concern. To address a concern with the sensitivity of the analytical measurements it was originally proposed to perform the study in two phases; in Phase I several measurements would be evaluated, while in Phase II the most sensitive measurement would be employed with a larger population. However, after the focus changed due to recruitment issues, the biological samples were collected from participants on a single sampling date for each community rather than in two phases as originally proposed.

The original Work was to include the following Tasks:

> **Task 1:** To investigate the utility of a Solid Phase Microextraction (SPME) method for extraction and analysis of VOC's in serum or urine.
>
> **Task 2:** To measure concentrations of metabolites of VOCs in serum or urine using LC-MS/MS (liquid chromatography-tandem mass spectrometry).
>
> **Task 3:** On a limited basis, to compare the results obtained from GC-MS (gas chromatography-mass spectrometry) analysis of serum and urine with results obtained using IM-MS/MS (ion mobilization-mass spectrometry) and measuring hemoglobin adducts in serum using LC-MS/MS.
>
> **Task 4:** Coordinate with performing parties in related studies for data collection, public participation, data analysis, public meetings for review of results and other ad hoc purposes.

After the change in focus, Work included the following changes in the Tasks:

> **Task 2:** There was inadequate blood collected to attempt to determine metabolites of the BTEX compounds using LC-MS/MS. Approval by IRB allowed for the collection of two, 10ml tubes of blood. One was used for VOC analysis and the other for DNA extraction. Due to the unstable nature of the measured VOCs, the VOC blood tube was only used for VOC analysis.
>
> **Task 3:** The IM-MS/MS was not performed for this project. The main reason being limited blood available for this work. Approval by IRB allowed for the collection of two, 10ml tubes of blood. One was used for VOC analysis and the other for DNA extraction. Due to the unstable nature of the measured VOCs, the VOC blood tube was only used for VOC analysis.
>
> **Task 5:** To utilize the ^{32}P-postlabeling assay to detect DNA adducts (both bulky and oxidative lesions) in human white blood cells (WBCs). DNA adducts are useful biomarkers of exposure and were added because of their utility to estimate exposures to complex environmental mixtures. DNA adducts (both bulky and oxidative lesions) were chosen to quantify potential exposures to the study population and to add additional insight to the proposed biomarker study.

Study Hypotheses

H_0: There will be no differences in concentrations of parent compounds of BTEX, 1,3-butadiene and bulky DNA adducts between a population residing in a community near major point sources of toxic air pollutants and a demographically similar population living in an area more distant from major sources of toxic air pollutants.

H_1: The population residing in a community near major point sources of toxic air pollutants would express higher concentrations of parent compounds of BTEX and 1,3-butadiene, and higher concentrations of bulky DNA adducts than a demographically similar population living in an area more distant from major sources of toxic air pollutants.

Study Design and Methods

Rational for selection of study areas

This study focused on two communities in Houston, Harris County, Texas; a study population in the Manchester neighborhood and a reference population in the Aldine neighborhood. Almost four million people reside in Harris County and share residence with the largest petrochemical complex in the country (Sexton et al. 2007). The Manchester and Aldine communities were chosen as the study and reference populations by the HEATS investigators (Figures 3, 4 and 5). In the HEATS final report (Morandi et al. 2009) the investigators give the following rational for the selection of the study areas: "The two areas were selected based on the differences in the number and type of TRI-reportable emission sources, similarities in sociodemographic characteristics, and the presence of at least one fixed monitoring site. The specific census tracts included in each area … were initially selected, in part, based on an analysis by the Texas Department of State Health Services that showed good matching of sociodemographic characteristics for most of these census tracks with regard to total population and important demographic characteristics such as ethnicity, age structure and income." Manchester is located in the Houston Ship Channel area, which is approximately 7 miles

southeast of downtown Houston. There are several industries bordering the Houston Ship Channel, specifically petroleum refineries and petrochemical industries, as well as highways, major shipping activities, and/or rail tracks. Aldine, the reference population, is located approximately 10 miles north of downtown Houston, removed from industrial sources of pollutants but near major highways.

TCEQ Fixed Site Ambient Monitoring Sites

TCEQ has the largest operating ambient monitoring network for hazardous air pollutants of any state in the U.S., with the Houston metropolitan area having the largest and longest running network of any metropolitan area in the U.S., including the entire state of California. Stationary sampling sites closest to the sampled neighborhoods include:

1) Manchester, which collects 24-hr canister samples of approximately 100 VOCs every 6th day.

2) Aldine, which collects 24-hr canister samples of approximately 100 VOCs every 6^{th} day.

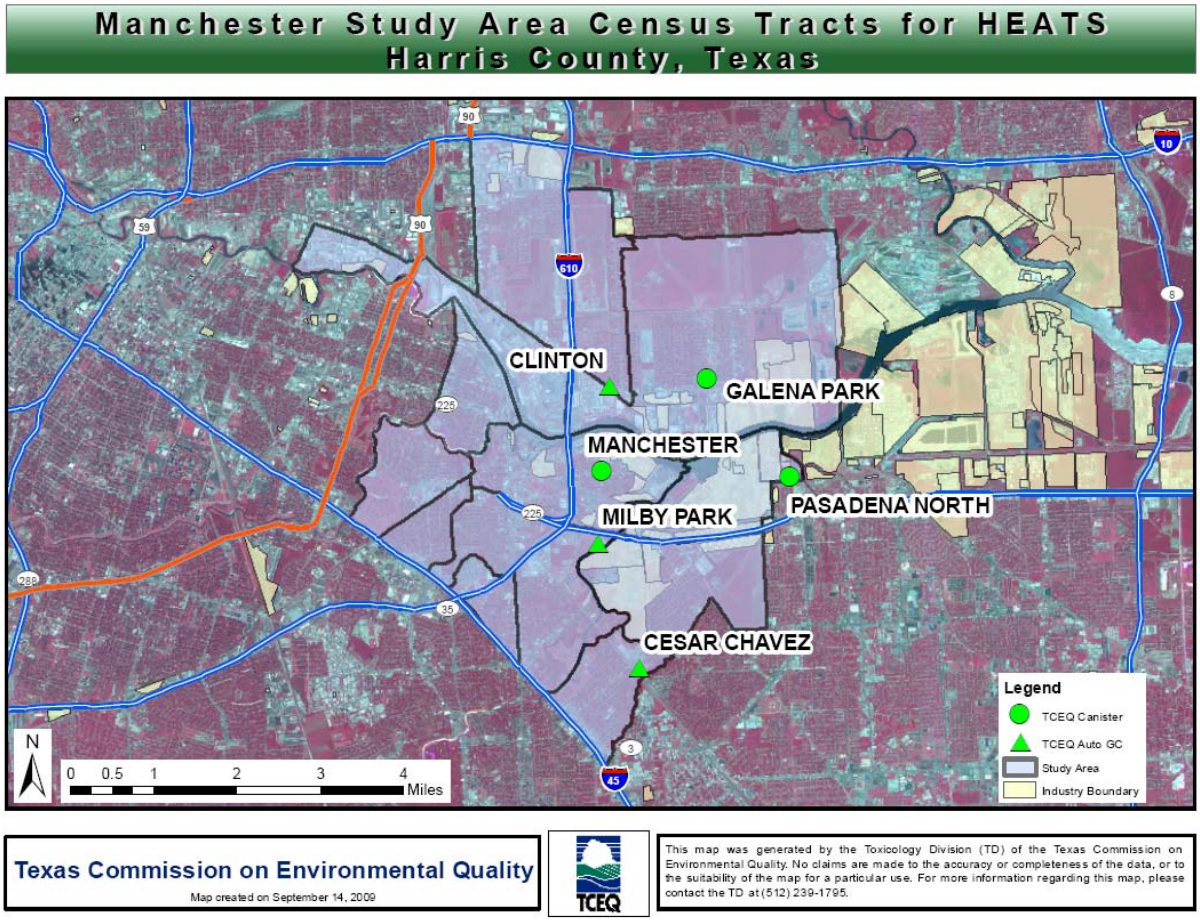

Figure 3. Map of the Manchester study area census tracts HEATS used for random selection participant recruitment. Map generated and provided by TCEQ.

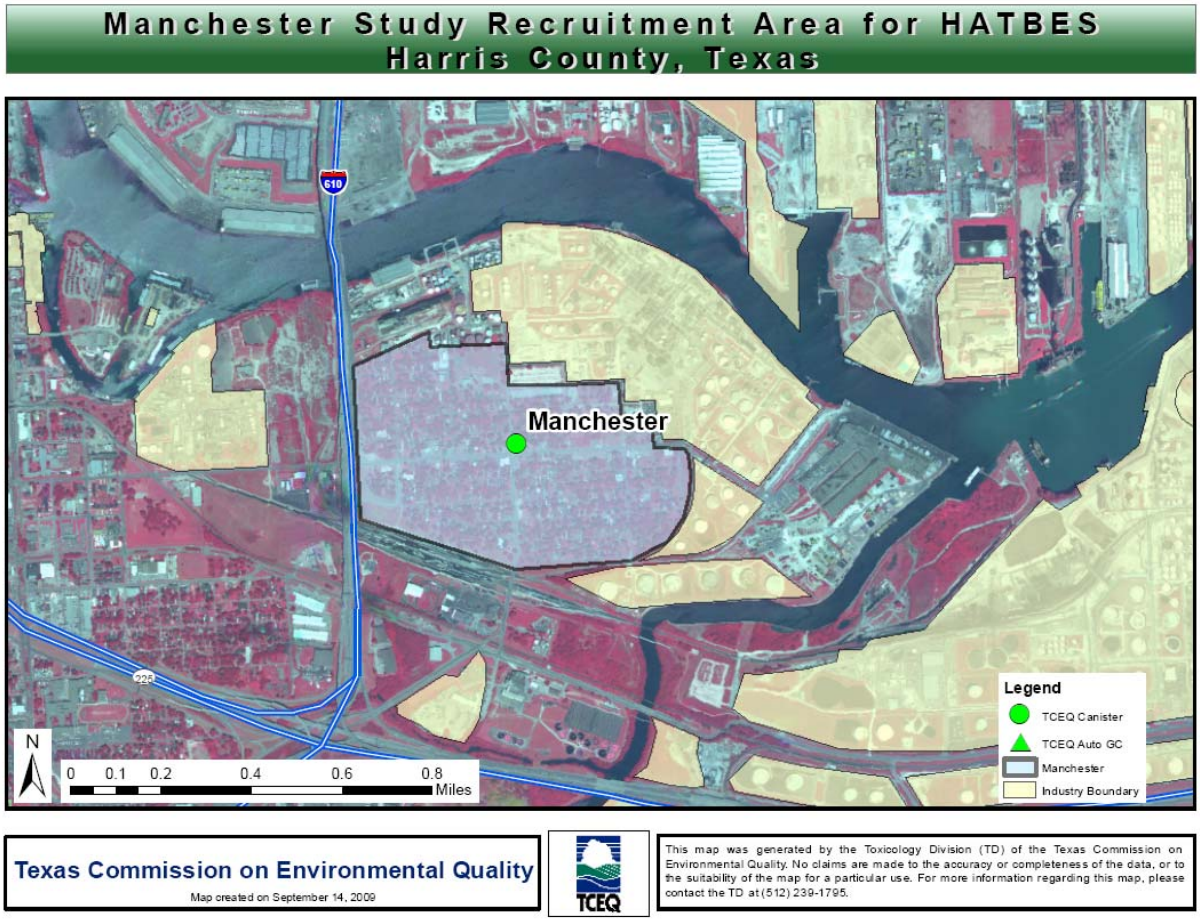

Figure 4. Map of the Manchester study area HATBES used for voluntary participant recruitment. Map generated and provided by TCEQ.

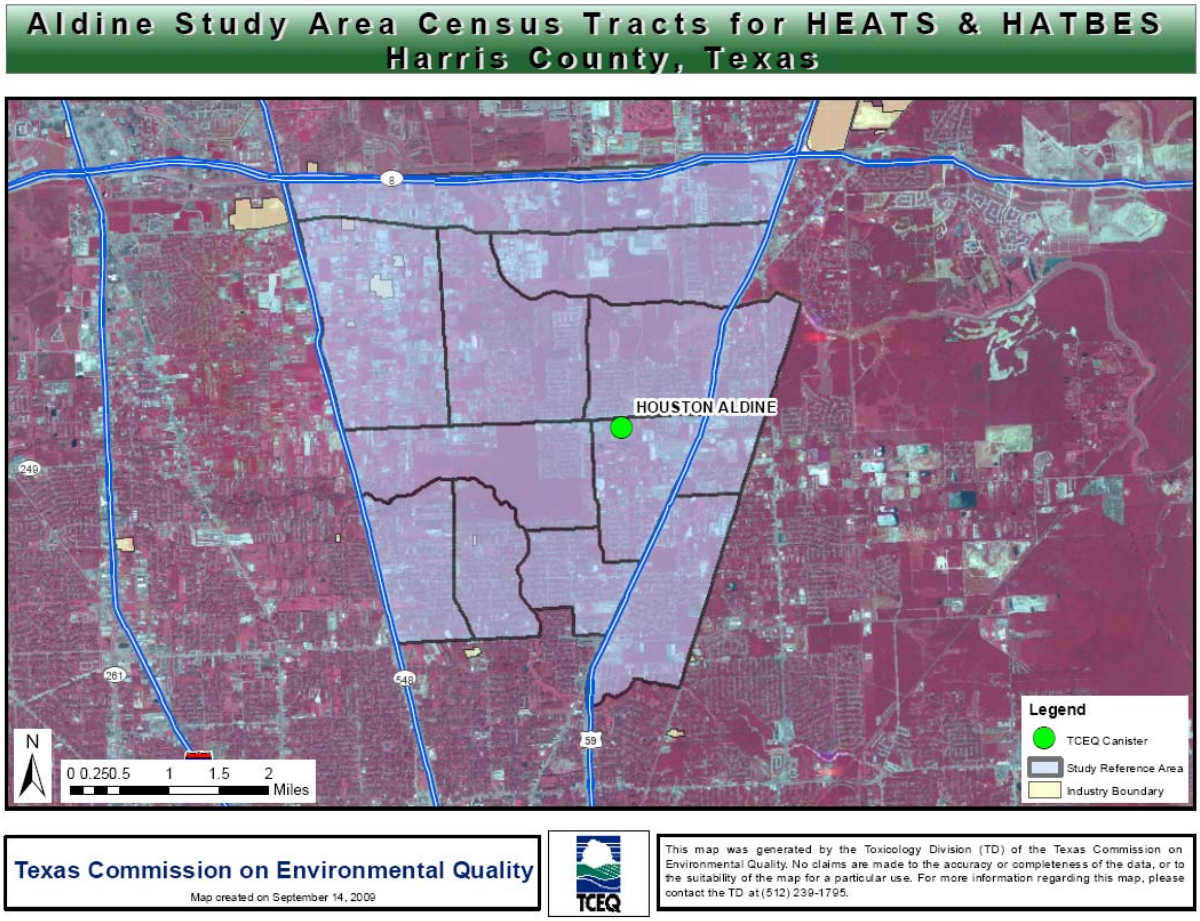

Figure 5. Map of the study area census tracts HEATS used for random selection participant recruitment. This is the same area HATBES used for voluntary participant recruitment. Map generated and provided by TCEQ.

Data Collection Methods

Participant Recruitment and Sample Collection

The initial study design anticipated partnership with HEATS. Through this collaboration, participants from the selected study areas would be randomly selected and recruited by HEATS and then given the opportunity to participate in HATBES. Unfortunately, due to unforeseen circumstances, which included difficulties with recruiting and retaining volunteers after Hurricane Ike, HATBES received permission from TCEQ to recruit from the general population. The majority of HATBES participants were recruited with the assistance of the Texas Environmental Justice Advocacy Services (TEJAS), with sample collection being independent in each community. Manchester sample collection took place in December 2008, and sample collection in Aldine was held in April 2009. The recruitment for these collections was facilitated by the TEJAS organization by spreading HATBES awareness though community functions, as well as a community meeting held by TAMU in the Manchester community. There were a total of ten participants recruited from HEATS (five from Aldine and five from Manchester).

Collection of samples from the HEATS participants were conducted at each individual's residence in August, September, October, and November; the remainder of the samples were collected at their respective community center during two individual sampling dates. All study participants were provided written informed consent prior to participating in the study. The consent form advised participants about the nature of the study, as well as the risks and benefits involved in participating in the study. All study forms and protocols were approved by the Institutional Review Board at TAMU prior to initiation of participant recruitment.

Blood and urine samples were collected from each participant during the respective sampling dates. Sample collection in Manchester was held in the community at the Fred Hartman Park community center on December 6, 2008. While the Aldine sample collection took place at the Thomas R. Wussow Park community center in the Aldine community on April 4, 2009. Each sample collection lasted approximately 4 hours at the respective community centers. Samples were stored on ice until shipped to the laboratory at TAMU for analysis. DNA was extracted from whole blood the following week, while VOCs were extracted and analyzed from whole blood and urine within the next month. Blood collection was administered by a phlebotomist from the Gulf Coast Research Center (GCRC). Blood samples were collected by venipuncture into two sterile glass 10 mL vacutainers containing sodium heparin (green top) and potassium oxalate/sodium fluoride (grey top). Green top vacutainers were used for DNA adduct analysis and grey top vacutainers were used for measuring VOC concentrations. Urine samples were collected by the participant in a sterile urine collection cup. Immediately after urine collection, 20 mL of urine was transferred by a study investigator into two 10 mL sterile plastic urinalysis vacutainers (yellow top) intended for chemistry urine testing.

Laboratory Analytical Methodologies

Cotinine Analysis

A Calbiotech-Cotinine Direct Enzyme Linked Immunosorbent Assay (ELISA) Kit intended for the measurement of cotinine in urine was used to determine smoking status. Cotinine, due to its longer half life than nicotine, has been used in research as a reliable marker for smoking status (Calbiotech Inc. 2009). The Calbiotech Cotinine Kit is a solid phase competitive ELISA. The urine samples and cotinine enzyme conjugate were added to the wells coated with anti-cotinine antibody. Urine samples, standards, and controls were run in duplicate and absorbance was read at 450nm with a FLUOstar Optima from BMG LABtech. Participants with urine cotinine levels greater than 17 ng/mL were classified as smokers as this was a natural break in cotinine levels, others were classified as non-smokers. This suggested natural cut-off point was where the largest difference was observed between two consecutive cotinine concentrations below 50 ng/mL in the analyzed urine samples. This point of reference is similar to the 14 ng/mL smoking status classification used by Lin et al. 2008.

Creatinine Analysis

Urinary creatinine concentrations were measured in order to correct for variations in urinary concentrations of environmental chemicals. Creatinine is a protein that is excreted from the body in urine at a fairly constant rate, and because of this, creatinine adjustment has been used to adjust urinary concentrations of environmental chemicals during exposure monitoring (Barr et al. 2004). Creatinine concentrations in individual urine samples were measured at St. Joseph's Regional Health Center Laboratory in Bryan, Texas using an auto-analyzer to allow

VOC values to be normalized for creatinine. The laboratory used an enzymatic method with the calibration traceable to the isotope dilution mass spectrometry and analyzed samples with an Ortho Vitros 5.1 fusion series analyzer. Urinary concentrations of BTEX and 1,3-butadiene were expressed as ng/mg creatinine.

Volatile Organic Compound Analysis

Unmetabolized BTEX and 1,3-butadiene in blood and urine were determined by headspace solid-phase micro-extraction/gas chromatography-mass spectrometry (HS-SPME/GC–MS). Immediately before analysis, 2 mL of blood was transferred by syringe into a sealed glass autosampler vial containing 8 mL deionized water. Urine samples of 5-10 mL were analyzed in 20 mL glass autosampler vials. Each sample was injected with 10 µl of internal standard containing toluene-d8 and chlorobenzene-d5 before analysis.

Samples were analyzed using gas chromatography-mass spectrometry (GC/MS) in electron ionization (EI) mode using a Thermo Trace gas chromatograph coupled to a Thermo DSQ-II mass spectrometer. Sample injections were performed using a Thermo Tri-Plus SPME/Liquid autosampler. Chemicals of concern were extracted from the headspace with a 65 µm polydimethylsiloxane fiber (Supelco, PA). The injector, MS-transfer line, and ion source were maintained at 200, 280 and 200°C, respectively. An Rxi-1ms fused silica capillary column (60 m, 0.25 mm id, 0.25 µm film thickness) with helium as the carrier gas was used for the separation of the analytes. The GC oven was maintained at 45°C for 9 minutes, and ramped at 10°C/min to 100°C. The mass spectrometer was operated with an electron impact energy of 70ev and the ions monitored were 53, 78, 91, 106, 98 and 117 (1,3-butadiene, benzene, toluene, ethylbenzene and xylenes, toluene-d8 and chlorobenzene-d5).

^{32}P-postlabeling DNA Adduct Analysis

DNA adducts were detected and quantified in human white blood cells (WBC) by using the nuclease P1 version of the ^{32}P- post-labeling assay (Reddy and Randerath 1986). The DNA was isolated by solvent extraction combined with enzymatic digestion of the protein and RNA. The DNA was dissolved in 1.5 mM sodium chloride, 0.15 mM sodium citrate, and each concentration measured spectrophotometrically at 260 nm. The isolated DNA was stored at -80°C until analysis. The nuclease P1-enhanced bisphosphate ^{32}P-postlabeling analysis was performed to determine the levels of DNA adducts in WBC. Ten µg of DNA from each sample was enzymatically degraded to normal (Np) and modified (Xp) deoxyribonucleoside 3′-monophosphates as well as dinucleotides containing 3'-terminal cA (Np-cAp) using micrococcal nuclease (Sigma) and spleen phosphodiesterase (Sigma) at pH 6.0 and 37°C for 3.5 hr. The hydrolyzed DNA was treated with nuclease P1 (Sigma), which selectively hydrolyzes normal 3′-mononucleotides to nucleosides, thereby enriching the mixture in modified (adducted) 3′-monophosphates. The enriched modified nucleotides were converted to 5′-^{32}P-labeled 3′,5′-bisphosphate derivatives by incubation with carrier-free [γ-^{32}P]ATP (MP) and polynucleotide kinase (USB) mediated phosphorylation. The ^{32}P-labeled DNA adducts were purified and partially resolved by one-dimensional development (D1) using 2.3M NaH_2PO_4 (pH 5.75). An original area of the lower (L, 2.8 x 1 cm) and central (C, 2.8 x 1 cm) portions of cellulose map (D1) containing adducts were excised and the ^{32}P-labeled products were contact-transferred to fresh polyethyleneimine (PEI)-cellulose sheets. Labeled products were then resolved by two-dimensional thin-layer chromatography (TLC). The first dimension used 3.82M lithium formate

+ 6.75M urea (pH 3.35). The second dimension was obtained using 0.72M $NaH_2PO_4 \bullet H_2O$ + 0.4M TRIS + 7.65M urea (pH 8.2).

The ^{32}P-labeled products or radioactive spots on each chromatogram were quantitated by InstantImager (PerkinElmer). Screen-enhanced autoradiography of the chromatograms was performed on Kodak XAR-5 X-ray (gray) film for 25 h at -80°C. The count rates of individual radioactive spots from each exposure group were used to calculate relative adduct labeling (RAL) values ± standard error of the mean (SEM) for each spot. RAL values were calculated according to the following equation:

RAL = Sample count rate [cpm] ÷ (DNA-P [pmol] * Spec. Act. $_{ATP}$ [cpm/pmol])

Where DNA-P represents the amount of DNA assayed (expressed as pmol DNA monomer units) and specific activity (Spec. Act.) of [γ-^{32}P]ATP used in the labeling reaction. Thus, for 10 µg DNA, and ATP with a specific activity of $4.5X10^6$ cpm/pmol, 145 cpm corresponds to a RAL value of 10^9 (i.e., an estimated level of one modification in 10^9 DNA nucleotides). Total RAL values were calculated by summing the values for individual spots from each DNA sample. Differences of mean DNA adduct levels between exposure groups were tested with an unpaired Student's *t*-test. The probability level used for determining significance was $P < 0.05$.

Results and Discussion

Demographics of Study Populations

A total of 104 participants were recruited from the two communities; 53 from Manchester and 51 from Aldine (Table 1). Of these 104 participants, 10 were recruited by HEATS (5 from Manchester and 5 from Aldine) during the months of August-November 2009. The most notable difference between subject participation in the communities were between ethnic groups. Participant recruitment in the Manchester community resulted in 100% participation by Hispanic ethnicities, where Aldine had participation by a more diverse population of Hispanic (72%), African American (24%) and Caucasian ethnicities (4%). There was a higher participation by females in both populations (~60%), and the average age of both genders was slightly lower in Aldine (41 years of age) than the average age in Manchester (46 years of age).

Houston Air Toxics Biomarkers of Exposure Study		
	Aldine (n=51)	**Manchester (n=53)**
Gender	**(%)**	**(%)**
Males	31	36
Females	69	64
Race	**(%)**	**(%)**
African American	24	0
Hispanic	72	100
Caucasian	4	0
Other	0	0
Age	**(Avg.)**	**(Avg.)**
Females	38	40
Males	44	51

Table 1. Demographics of the HATBES Participants (Including the 10 participants recruited by HEATS)

TCEQ Ambient Air Monitoring Data

The TCEQ provided air sampling data for specific VOCs from the stationary every 6th day 24-hour canister monitors located in Aldine and Manchester. Evaluation of VOC concentration in ambient air was completed by taking a 12-month rolling average from April 2008 through March 2009, as well as identifying a one day air samples obtained prior to sample collection in the Manchester and Aldine communities from the TCEQ ambient air monitors in each location. Mean concentrations for all measured VOCs in Manchester were higher than the mean concentration of VOCs in Aldine for both the 12-month rolling averages (Tables 2 and 3) and the concentrations from the air samples taken prior to each sample collection (Figure 6). Although it should be noted that all concentrations reported were lower than their respective one-hour TCEQ guideline for acceptable acute exposure (Tables 2 and 3). For long-term screening, the TCEQ has set a level for benzene in ambient air of 1.4 ppb_v, which falls within EPA's recommended risk range. Details of the derivation of the TCEQ benzene screening numbers can be found in the TCEQ Development Support Document for Benzene (TCEQ 2007). TCEQ guidelines for acceptable acute and chronic ambient air exposure can be found on TCEQ's public website (http://www.tceq.state.tx.us/implementation/tox/AirToxics.html). In the case of benzene, rather than calculating one number per risk level, as is typically done, the EPA has set a risk range for looking at chronic benzene exposure. EPA states in their IRIS benzene support document that "A range of estimates of risk is recommended, each having equal scientific plausibility" (EPA 2003). For a risk level of 1 in 100,000, the level used by the TCEQ for the State of Texas, the range EPA recommends is 0.4 – 1.4 ppb_v. Since the long-term

screening values are set to protect against long-term exposure, a proper comparison for them is with at least one year of data. A 12-month rolling average was calculated from April 2008 through March 2009 data from the Manchester and Aldine ambient air monitors. The 12-month rolling averages are well below the TCEQ long-term screening value and are within the acceptable range EPA has developed for a risk level of 1 in 100,000 (Tables 2 and 3).

Meteorological data was obtained from TCEQ stationary monitors located in the Manchester and Aldine communities. Meteorological data, including wind direction, wind speed and temperature, is logged every hour during a 24 hour time period. In order to make comparisons to the one day air monitoring data reported prior to sample collection, meteorological data was chosen from the same day. On December 2, 2008 in the Manchester community, the average wind direction was from the South Southeast (163 Degrees) at 6 miles per hour, while the average temperature was 57 degrees Fahrenheit. In Aldine on April 1, 2009 the average wind direction was from the East (100 Degrees) at 7 miles per hour, while the average temperature was 63 degrees Fahrenheit.

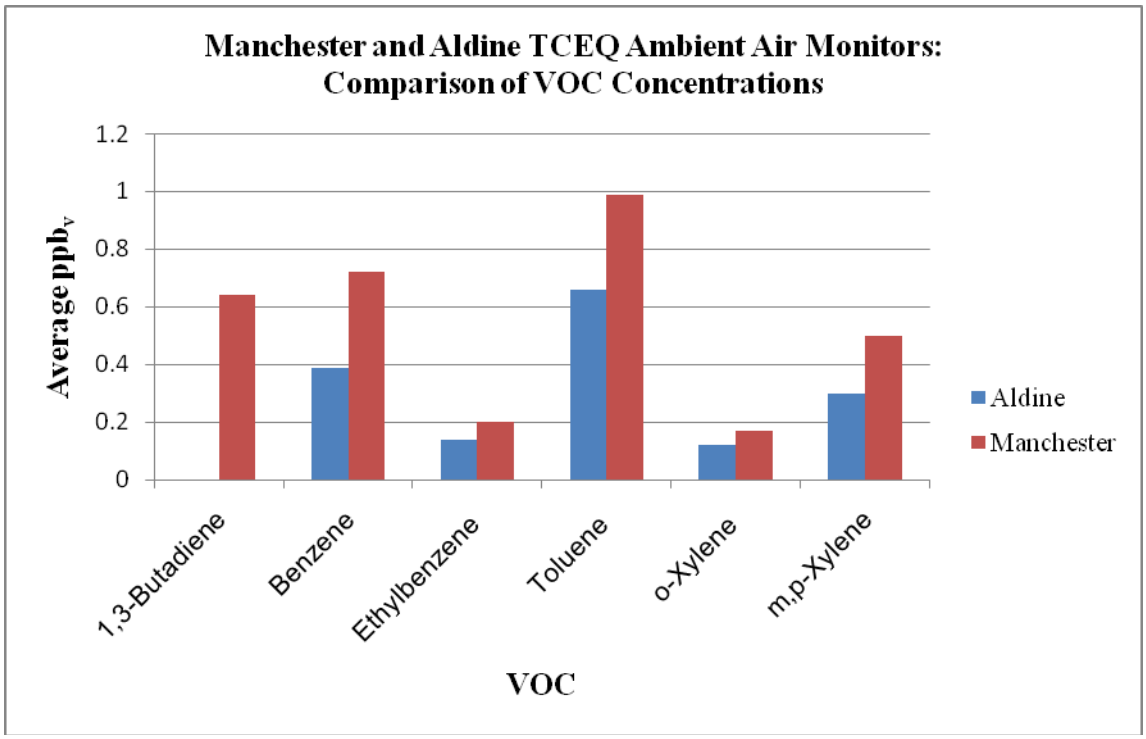

Figure 6. TCEQ 24-Hour canister sampling data for VOCs from the Manchester and Aldine communities. Air samples were taken prior to each sample collection in Manchester (Date sampled:December 2, 2008) and Aldine (Date sampled: April 1, 2009).

Table 2. Comparison of 12-month rolling average and max concentrations of 24-hour ambient air concentrations measured at the TCEQ Manchester ambient air monitor every 6 days during April 2008 through March 2009.

Manchester ambient air monitor: Comparison of 12-month rolling average and max values to TCEQ Appropriate Screening Values (ACVs)				
VOC	**12-month Rolling Average (ppb_v)**	**Long-Term TCEQ Screening Value (ppb_v)**	**Max Ambient Concentration (ppb_v)**	**Short-Term TCEQ Screening Value (ppb_v)**
1,3-Butadiene	0.35	9.1	2.04	1700
Benzene	0.71	1.4	3.63	180
Ethylbenzene	0.23	100	0.56	1000
Toluene	1.02	1100	3.75	4000
o-Xylene	0.19	140	0.53	1700
m,p-Xylene	0.55	140	1.45	1700

Table 3. Comparison of 12-month rolling average and max concentrations of 24-hour ambient air concentrations measured at the TCEQ Aldine ambient air monitor every 6 days during April 2008 through March 2009.

Aldine ambient air monitor: Comparison of 12-month rolling average and max values to TCEQ Appropriate Screening Values (ACVs)				
VOC	**12-month Rolling Average (ppb_v)**	**Long-Term TCEQ Screening Value (ppb_v)**	**Max Ambient Concentration (ppb_v)**	**Short-Term TCEQ Screening Value (ppb_v)**
1,3-Butadiene	0.12	9.1	0.72	1700
Benzene	0.50	1.4	2.14	180
Ethylbenzene	0.17	100	0.96	1000
Toluene	0.83	1100	4.48	4000
o-Xylene	0.15	140	1.04	1700
m,p-Xylene	0.44	140	2.99	1700

VOC Analysis

The distributions of VOC concentrations in blood (median ± standard deviation (SD)) and urine (mean ± SD) are shown in Tables 4 and 5. Benzene and m,p-xylene, in which 87% and 71%, respectively, of measurements were below the reporting limit, were the only analytes observed above the detection limit in the Manchester blood samples. Reporting limits are reported as two times the method detection limit. In Aldine, benzene was the only detectable analyte in blood, with 84% of measurements below the reporting limit. All urine VOCs concentrations were reported after adjusting for creatinine levels. Overall detection limits for VOCs in blood and urine are reported in Table 6.

Evaluations of benzene blood and urine concentrations (reported by individual concentrations) are shown in Figure 7. Urinary concentrations of BTEX and 1,3-butadiene were expressed as ng/mg creatinine in order to correct for variations in urinary concentrations of environmental chemicals. The Aldine community had 8 participants and Manchester had 6 participants with blood benzene concentrations above the reporting limit of 0.56 ng/mL (Table 7). In contrast, urinary benzene concentrations, after adjusting for creatinine, showed Aldine did not have any participants with urine benzene levels greater than 1 ng/mg creatinine, where as Manchester had 4 participants with urine benzene levels above 1 ng/mg creatinine (Table 7). Out of the 10 HEATS participants, only one participant from Aldine had detectible levels of VOCs in blood. This participant had a blood benzene level of 1.11 ng/mL.

Comparisons of blood and urine benzene levels between the two communities and separated by smoking status were assessed using two-way analysis of variance (ANOVA) statistical analysis in SPSS Version 16.0 (SPSS Inc., Chicago, Illinois, USA) and are shown in Table 8 and Table 9. No significant differences in benzene levels were found between the two communities, and no significant differences were found between smoking status for blood and urine benzene concentrations.

Table 4. Blood VOC Concentrations, BRL= Below Reporting Limit, Median ± SD, reported in ng/mL, percent above reporting limit.

Blood VOC Concentrations		
VOC	**Manchester N= 48**	**Aldine N= 50**
1,3-Butadiene	BRL	BRL
Benzene	0.28 (0.34)	0.28 (0.85)
Toluene	BRL	BRL
Ethylbenzene	BRL	BRL
m,p-Xylene	0.98 (0.93)	BRL
o-Xylene	BRL	BRL

Table 5. Urine VOC Concentrations (ng/mL), Mean ± SD), reported in ng/mg creatinine.

Urine VOC Concentrations		
VOC	**Manchester N= 52**	**Aldine N= 48**
1,3-Butadiene	4.24 ± 12.16	1.49 ± 0.28
Benzene	0.75 ± 4.23	0.07 ± 0.01
Toluene	0.11 ± 0.14	0.10 ± 0.02
Ethylbenzene	0.66 ± 1.17	0.82 ± 0.25
m,p-Xylene	0.83 ± 1.18	2.43 ± 0.35
o-Xylene	1.22 ± 2.01	2.03 ± 0.56

Table 6. VOC method detection limits (ng/mL) for blood and urine samples using the HS-SPME/GC–MS method.

VOC Method Detection Limits		
	Blood	Urine
1,3-Butadiene	6.79	1.36
Benzene	0.28	0.06
Toluene	0.44	0.09
Ethylbenzene	0.53	0.07
m,p-Xylene	0.98	0.20
o-Xylene	0.47	0.09

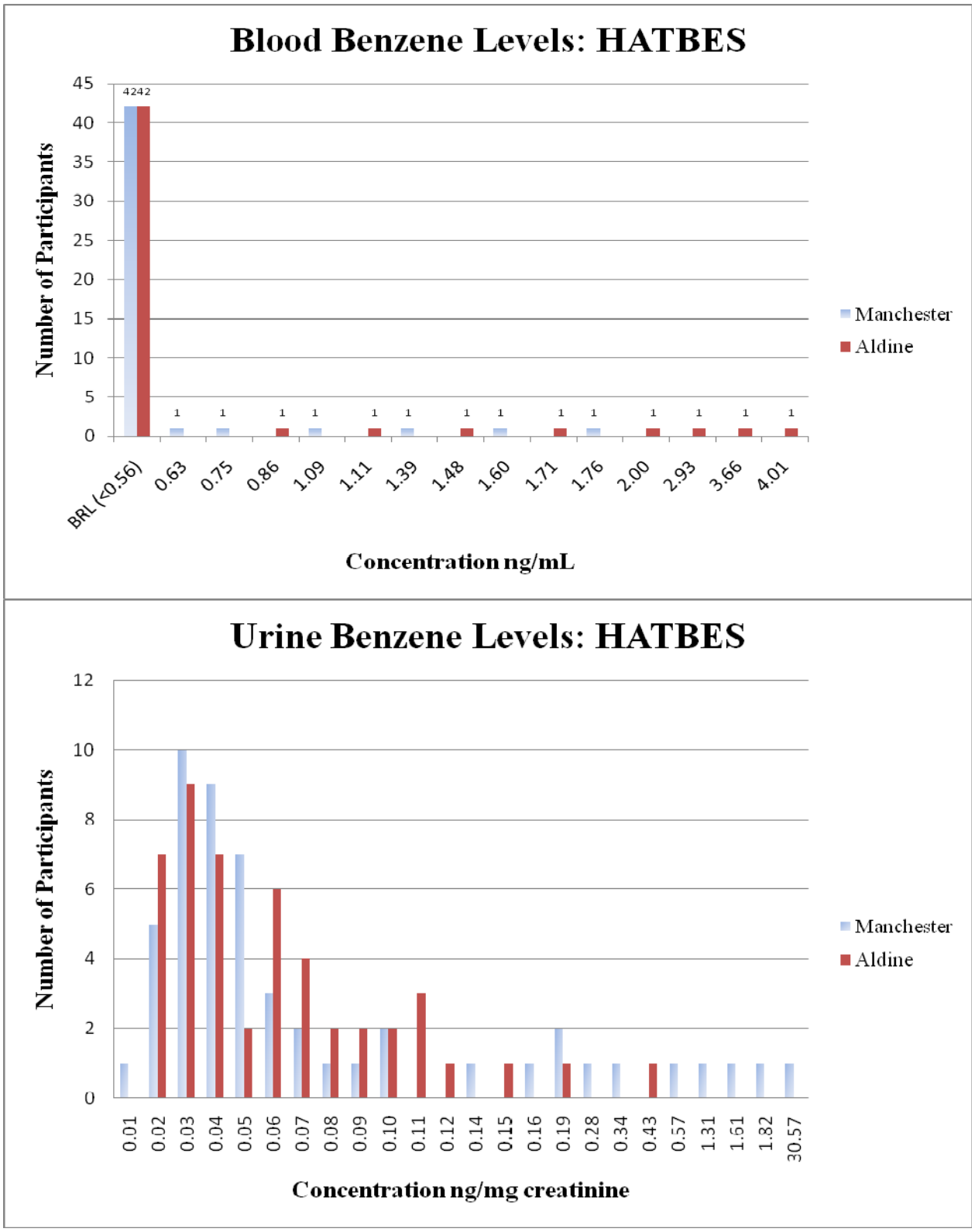

Figure 7. Blood and urine benzene concentrations in Manchester and Aldine (urinary concentrations of BTEX and 1,3-butadiene are expressed as ng/mg creatinine).

Table 7. Individual blood (ng/mL) and urine (ng/mg creatinine) benzene concentrations.

Blood Benzene Levels	
Concentrations Above Reporting Limit (>0.56 ng/mL)	
Manchester (n= 6)	**Aldine (n=8)**
0.63	0.86
0.75	1.11
1.09	1.48
1.39	1.71
1.60	2.00
1.76	2.93
	3.66
	4.01
Urine Benzene Levels	
Concentrations Above 1 ng/mg creatinine	
Manchester (n= 4)	**Aldine (n=0)**
1.31	
1.61	
1.82	
30.57	

Table 8. Blood Benzene Concentrations by Exposure Group and Smoking Status

Exposure Group	Smoking Status	Mean (ng/mL)	Std. Deviation	N
Manchester	Smoker	0.28	0.00	10
	Non-Smoker	0.43	0.38	38
	Total	0.40	0.34	48
Aldine	Smoker	0.34	0.19	9
	Non-Smoker	0.64	0.92	41
	Total	0.59	0.85	50
Total	Smoker	0.31	0.13	19
	Non-Smoker	0.54	0.72	79
	Total	0.49	0.65	98

Table 9. Urine Benzene Concentrations by Exposure Group and Smoking Status

Exposure Group	Smoking Status	Mean (ng/mg cr)	Std. Deviation	N
Manchester	Smoker	0.10	0.16	11
	Non-Smoker	0.93	4.76	41
	Total	0.75	4.23	52
Aldine	Smoker	0.03	0.01	8
	Non-Smoker	0.07	0.07	40
	Total	0.07	0.07	48
Total	Smoker	0.07	0.13	19
	Non-Smoker	0.50	3.40	81
	Total	0.42	3.06	100

DNA Adducts

Initially, bulky DNA adducts (ie. adducts caused by heavy molecular weight compounds) in WBC were detected by ^{32}P-postlabeling assay in the Manchester (n=20) and Aldine (n=20) communities. Results displayed very low levels of adducts and there were no significant differences of adduct levels in the two communities. It has been suggested that a ^{32}P-postlabeling assay that detects oxidative DNA lesions could be more useful, considering VOCs are the hypothesized exposures in the study area (Randerath et al. 2001). Benzene exposure can occur in many settings including residential and industrial, and the most important chronic adverse effect of benzene exposure is hematopoietic toxicity. The mechanism for toxicity is not completely understood, but it appears that oxidative stress through ROS formation plays a crucial role in toxicity (Casarett et al. 2008). Is a known that benzene is bioactivated into its major metabolites (ie. phenol, hydroquinone, and catechol). These metabolites bind covalently to DNA and induce intracellular effects such as DNA damage by ROS leading to carcinogenesis. Catechol forms more reactive semiquinones and reactive quinones that are thought to play an important role in the generation of ROS (Barreto et al. 2009). The oxidative DNA damage caused by VOCs can be measured by type II I compounds by the P1-enhanced version of the ^{32}P-postlabeling assay (Randerath et al. 2001, Zhou et al. 2004).

Samples from the Manchester (n=16) and Aldine (n=16) communities were chosen based on non-smoker status and matched by gender and age. Oxidative DNA lesions in WBC were detected by ^{32}P-postlabeling assay. The profiles of oxidative DNA adducts on PEI-cellulose maps are qualitatively similar (Figure 8). The adducts measured are chromatographically similar to *in-vitro* oligonucleoties containing cA found by Randerath et al. 2001. Levels of type II I-compounds containing 8,5'-cyclo-2'-deoxyadenosine adducts (CcA, TcA and GcA) in WBC of the participants living in Manchester were higher compared to those in Aldine (Figure 9 and Table 10). A Student's *t*-test was used to determine the significant differences between the two communities. Mean relative adducts for individual spots, CcA, TcA and GcA displayed higher levels in Manchester than in Aldine (Table 10); P values were 0.078, 0.018, and 0.061 for CA, TA, and GA, respectively. The total levels of three oxidative DNA damage products (CA, TA, GA) showed a significant difference between Manchester and Aldine ($P<0.05$). The results for these comparisons can be found in Figure 9 and Table 10.

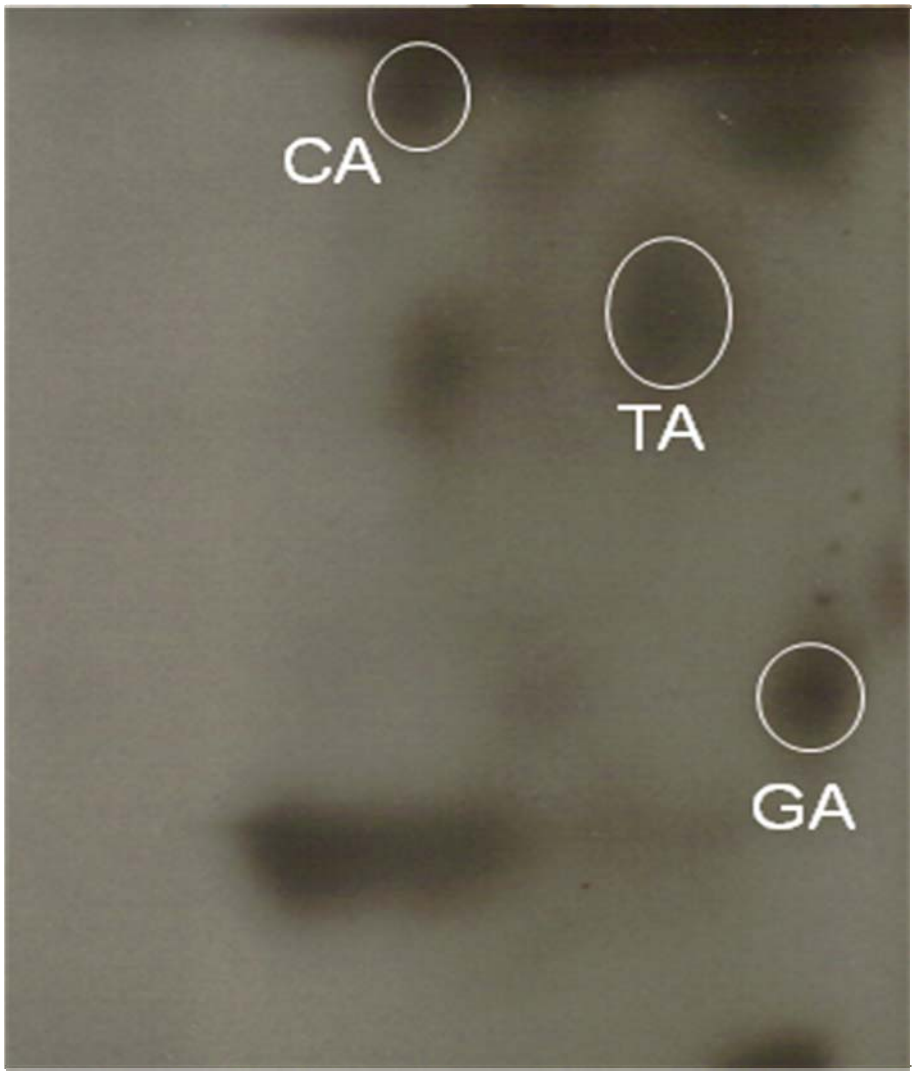

Figure 8. Representative profiles from TLC of ^{32}P-postlabeled type II I-compounds in central map from human WBC DNA. Circled spots are type II I-compounds CA, TA, GA which represent biomarkers of oxidative DNA damage.

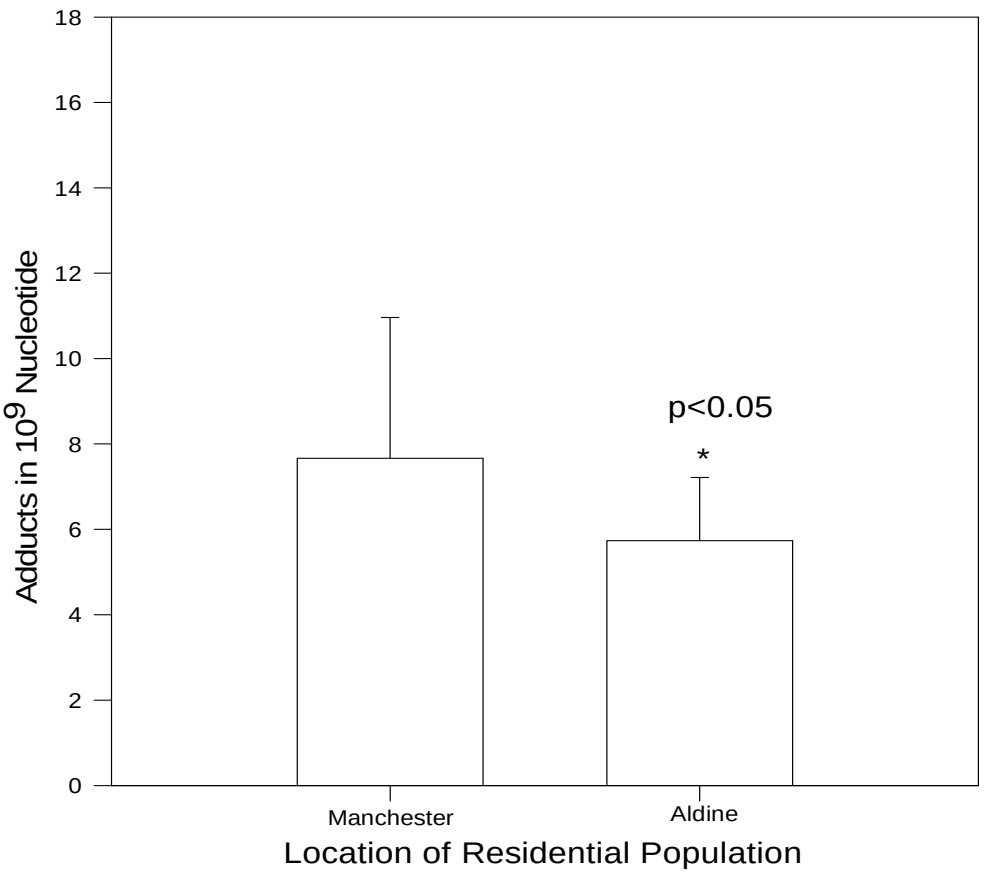

Figure 9. Comparison of total oxidative DNA lesions in human WBC between the Manchester and Aldine communities with standard error bars (* Significant difference P-Value <0.05).

Table 10. Comparison of Oxidative DNA Adducts* in Human WBC in the Manchester and Aldine Communities. *P-Values determined using a Student's t-test.

Comparison of Oxidative DNA Adducts in Manchester and Aldine							
Adduct	Manchester (Study Population)			Aldine (Reference Population)			P*
	n	Mean	SEM	N	Mean	SEM	
CA	16	2.79	0.27	16	2.22	0.16	0.078
TA	16	1.91	0.2	16	1.34	0.11	0.018
GA	16	2.81	0.27	16	2.18	0.18	0.061
Total	16	7.67	0.82	16	5.74	0.37	0.041

Conclusions

In this study on biomarkers of exposure to VOCs (1,3-butadiene and BTEX), the null hypothesis proved correct: There will be no differences in concentrations of parent compounds of BTEX, 1,3-butadene and bulky DNA adducts between a population residing in a community near major point sources of air pollutants and a demographically similar population living in an area more distant from major sources of air pollutants. This conclusion is supported by the TCEQ air monitoring data collected in the communities of Manchester and Aldine, which show similar levels of airborne contaminant concentrations for these VOCs. There were no significant differences detected between ambient air exposures or biomarkers of exposure to parent VOC compounds in the two residential communities. It was also determined that the rolling 12-month averages of benzene in air reported from the TCEQ Manchester and Aldine ambient air monitors are well below the TCEQ long-term screening value and are within the acceptable range EPA has developed for the 10^{-5} risk level (0.4 – 1.4 ppb_v).

While the median blood benzene levels from both communities were above the levels found in the NHANES US residential population, it is impossible to draw conclusions about this comparison as the blood benzene means for the HATBES study populations are both at the analytical detection limit for this study, which is higher than the analytical detection limit for NHANES. Because of the differences in detection limits, it is impossible to determine if the HATBES data mean is actually above the NHANES data mean, or if in reality they are similar. A much lower detection limit would need to be achieved to compare this dataset to NHANES. Even though blood concentration levels were higher when compared to the NHANES data, all of the detected benzene concentrations fall below occupational levels found in petrochemical workers in Italy (Table 11).

It is unknown why there is a significant difference between total oxidative DNA lesions in human WBCs between the Manchester and Aldine communities, although these results may provide a focus for future research on differences in oxidative DNA lesions in human WBCs.

It should be noted that there was a non-significant higher concentration of benzene found in the blood and urine of non-smokers compared to smokers. Although the results are not significantly different, this is not typically observed in benzene biomarker studies. These results could be due to the overall low levels of benzene exposure and the concentration at which the laboratory method detection limit was set for benzene.

The results from this study indicate these populations were exposed to VOCs and the methods used to quantitiate biomarkers of exposure are sufficient to detect residential level exposure to these compounds. However, they are not yet low enough to properly compare with other datasets such as the NHANES dataset. Due to the lack of significant differences in the concentrations of monitored VOCs between these two residential communities it appears that the petrochemical industries near the Manchester community are not the main source of VOC exposure. It also appears that there is a more complex contribution of air pollutants from numerous urban sources, such as, automobile traffic, airports, railroad activities, construction, and other environmental and lifestyle exposures.

Table 11. Study comparisons between blood benzene levels (ng/mL) using the HS-SPME/GC–MS method. [a]Geometric Mean and Inter-quartile Ranges, [b]Reporting the minimum at the laboratory method detection limit

Population	Blood Benzene (ng/mL)	Smoking Status	Reference
General US (n=788)	0.05 (0.02-1.40)	NS	NHANES 2003
General US (n=265)	0.09 (0.06-0.12) [a]	NS	NHANES 2000
Petrochemical Workers Italy (n=86)	0.40 (0.10-13.58)	NS	Hoet 2008
HATBES Study Population [b] (n=48)	0.28 (0.30-1.76)	NS/S	TAMU 2009
HATBES Reference Population [b] (n=50)	0.28 (0.30-4.01)	NS/S	TAMU 2009

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