ASSESSING TARGETED GENOMIC EXPRESSION FOLLOWING IN VITRO EXPOSURES OF HUMAN LUNG CELLS TO AMBIENT GASES IN HOUSTON, TEXAS

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ABSTRACT

Hang Nguyen: Assessing targeted genomic expression following *In Vitro* exposures of human lung cells to ambient gases in Houston, Texas
(Under the direction of William Vizuete)

Current *in vitro* studies do not typically assess the cellular impacts in relation to ambient atmospheric mixtures of gases that are constantly undergoing chemical transformations. In the present study, we set out to examine the biological (i.e. mRNA) responses of human lung cells upon exposure to air toxics by comparing the expression in response to controlled ozone, clean air, and mixtures of gases found in the ambient air. These ambient exposures are the first testing of a field-deployed lung cell experiment. Examining mRNA levels, we identified changes in genes that play a role as inflammatory responders in the cell. These results highlight that cells exposed to clean air had minimal transcriptional change, while as anticipated, cells exposed to the ambient conditions displayed changes in 11 genes. The potential to produce field gas-phase toxicity data would enable environmental pollution researchers to begin to reduce uncertainties in gas exposure estimates.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALOX5	Arachidonate 5-Lipoxygenase
BEE-TEX	Benzene and other toxics exposure
C2	Complement Component 2
CAMS	Continuous ambient monitoring station
CCL11	Chemokine (C-C Motif) Ligand 11
CCL24	Chemokine (C-C Motif) Ligand 24
DEFA1	Defensin, Alpha 1
EPA	Environmental Protection Agency
FDR	False discovery rate
FGFR3	Fibroblast Growth Factor Receptor 3
GIVES	Gas Phase In Vitro Exposure System
HAPs	Hazardous air pollutants
HARC	Houston Advanced Research Center
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
IL8	Interleukin 8
IL11	Interleukin 11
IL12B	Interleukin 12B
LDH	Lactate dehydrogenase
MX2	MX Dynamin-Like GTPase 2
PTGIR	Prostaglandin I2 (Prostacyclin) Receptor
PTRMS	Proton-transfer-reaction mass spectrometer
TCEQ	Texas Commission Environmental Quality

UH	University of Houston
VOCs	Volatile organic carbon

CHAPTER 1: INTRODUCTION

In the ambient environment, the public can be exposed to a wide number of different gaseous air pollutants. These ambient exposures may include hazardous air pollutants (HAPs) such as benzene and 1, 3 butadiene, which are known carcinogens and are emitted by human activity directly into the atmosphere [1]. Other harmful pollutants, such as ozone, are not emitted but rather formed via chemical reactions that occur in the atmosphere. The resulting public exposure in the ambient environment is a constantly changing mixture of primary and secondary gas-phase pollutants that are influenced by both chemical transformations and physical processes. The dynamic nature of this exposure environment makes it a challenge to simulate in a laboratory setting and thus has limited our ability in risk assessment to a real-world mixture. The assessment of toxicity for many gas-phase pollutants, such as those found on the U.S. Environmental Protection Agency (EPA) Hazardous Air Pollutant (HAP) list, is based on evidence provided by exposures to a single pollutant [1]. Experiments to single pollutants are unable to assess any synergistic impact that may occur from the simultaneous exposure of multiple pollutants. Further, atmospheric chemistry produces a diverse number of different gas-phase species, many of which are not even measured but could be a contributor to the overall toxicity of an ambient exposure. The enormity of a number of species in the atmosphere and its dynamic nature require a systematic approach to identify what gas-phase species in an ambient exposure are the most critical to public health.

There has been limited exposure data generated that has quantified the risk due to exposures to mixtures of gas-phase pollutants [2,3]. In one study, the influence of atmospheric chemistry on emitted gas-phase pollutants was quantified using in vitro exposures to epithelial lung cells [4]. These researchers first exposed A549 epithelial lung cells to two primary emitted pollutants of 1,3 butadiene and isoprene, and then exposed their photochemically aged gas-phase products. After the exposures, biological responses were quantified using IL8 and LDH as biomarkers. When cells were exposed to major oxidation products there was up to 15-fold increase in LDH levels, and a 2-fold increase in IL8 levels when compared to exposures to just the primary pollutants [5]. Studies investigating oxidation products have also focused on urban exposures and their photochemical gas-phase products [6-9]. This work was completed through the in vitro exposures of A549 epithelial lung cells to an urban mixture of volatile organic carbon (VOC) and oxides of nitrogen (NOx) [9]. This urban mixture was created to represent an average U.S. city and consists of 55 different hydrocarbons [9]. In addition to in vitro exposures to this mixture, these researchers then photochemically aged these pollutants in an outdoor smog chamber and created secondary products that were also exposed to A549 cells. After exposure, the RNA was collected and then extracted and genomic responses were quantified for 28,869 genes using Affymetrix GeneChip® Human Gene 1.0 ST arrays (Affymetrix Inc., Santa Clara, CA) [9]. Exposure to the non-photochemically aged pollutants altered 19 genes while exposure to the photochemically aged pollutants altered over 700 genes. These studies show the importance of combined exposures of gas-phase pollutants and the substantial influence due to photochemistry in the ambient environment in epithelial lung cells.

In laboratory-based experiments, all exposure environments are a simulation of the real atmosphere. Exposures to the real atmosphere are needed to corroborate the results observed in the

laboratory and also to guide new experiments. A recent field campaign provided an opportunity to do in vitro exposures to a real atmosphere in the city of Houston, Texas. Houston is the fourth largest city in the US and features a large refining and petrochemical industry. A recent task force found that the resulting emissions in Houston put city residents at risk due to exposures to ozone and nine hazardous air pollutants [3]. To assess the exposure to gas-phase pollutants, the Houston Advanced Research Center (HARC) funded a field campaign called the Benzene and other Toxics Exposure (BEE-TEX) [10]. BEE-TEX study aimed at monitoring air quality in three neighborhoods in Houston Ship Channel, Texas through the use of real-time monitoring equipment and advanced remote sensing equipment.

The location, campaign infrastructure, and advanced measurements provided by the BEE-TEX study created an ideal opportunity to deploy an in vitro instrument into the field for real atmosphere exposures to epithelial lung cells. In February of 2015, A549 cells were exposed in vitro to positive and negative controls as well as ambient air at the field site on 9700 Manchester Street (29°43′00.18″N, 95°15′21.83″W) [11]. Results from the positive and negative exposures supported the production of quality assured data. Further, the cells had a significant genomic expression of 11 genes related to inflammatory, immunity and oxidative stress. This successful in vitro deployment and the amount of highly temporally resolved chemical characterization data present a unique opportunity to correlate pollutant exposures to specific gene expressions [9]. This study provides the first test of a field-deployed lung cell experiment to examine the genomic response of lung cells to complex mixtures of air pollutants.

CHAPTER 2: METHOD

1. Study site

Houston is the largest city in the Southern United State, the most populous city in Texas, and the fourth most populated metropolitan statistical area in the United States. With an estimation of 2013, the city population is 2.19 million people living in a land area of 599.6 square miles (1,553 km^2). Houston is home to more than 3,700 energy-related businesses, the Institute for Energy Research, Energy Research Park, and Advanced Energy Consortium. Houston is considered as the Energy Capital of the world. It is the location of 40 of the nation's 145 publicly traded oil and gas exploration and production firms, including 11 of the top 25 as ranked by 2011 total assets. Petrochemical industry produces the more important plastics and resins from raw materials. The Houston metropolitan area dominates U.S. production of three major resins: polyethylene, with 36.6% of U.S. capacity; polypropylene with 51.7%; and polyvinyl chloride with 41.5% [12]. The Houston Ship Channel in Houston, Texas is part of the Port of Houston, which ranks first in the United States in international waterborne tonnage handled, second in total cargo tonnage handled and the tenth largest port in the world. The Port handled 220 million short tons of domestic and foreign cargo in 2010. The channel is the host for big vessels between Houston-area and the Gulf of Mexico and serves an increasing volume of inland barge traffic [13].

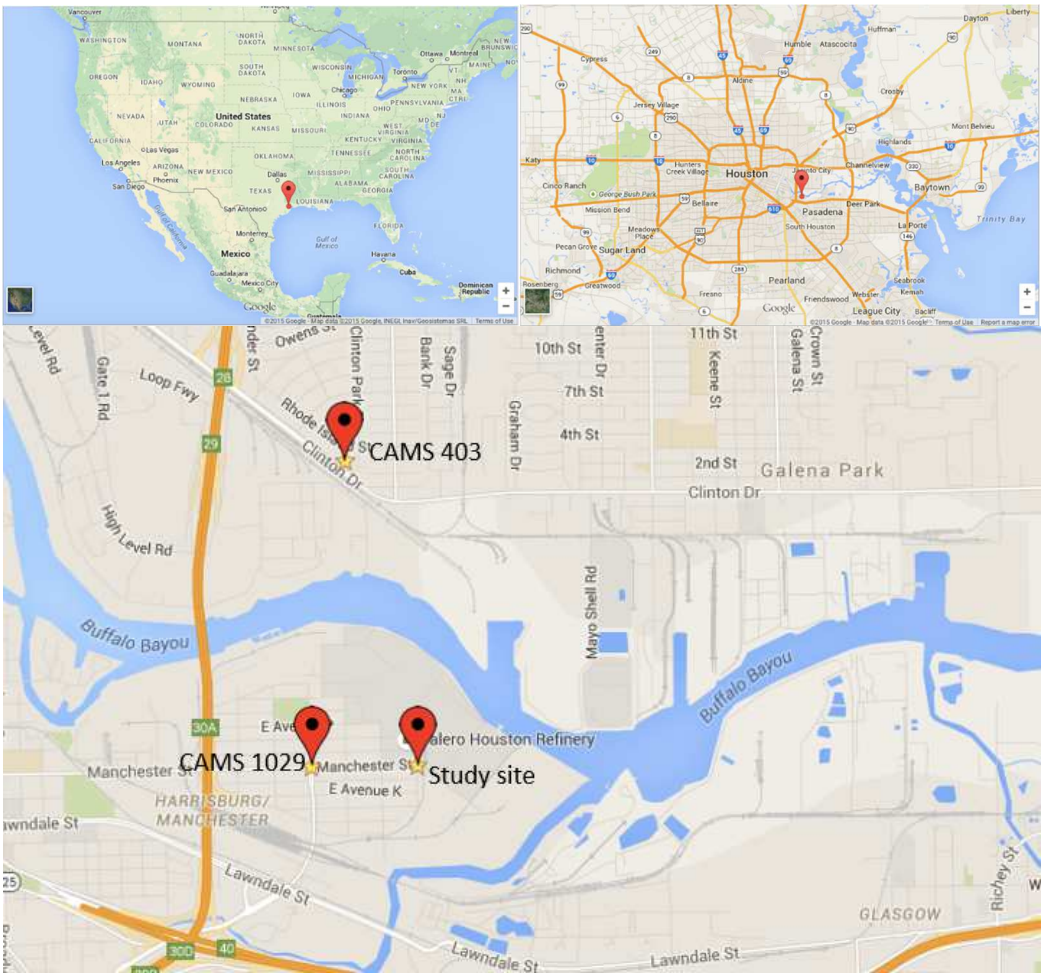

Figure 1: Location of study site for cell exposures, CAMs 1029 and CAMs 304

The study site is located at 9700 Manchester Street in downtown Houston, Texas, USA (29º43’00.18’’N, 95º15’21.83’’W). To the North and Northeast, Valero Houston refinery is across Manchester Street, as shown in Figure 1. 450 meters to the Northeast of the site is the watercourse of Buffalo Bayou Channel. To the Southeast, it is around 545 meters far from Sims Bayou Channel. Approximately 1.2 kilometers to the East is the E loop Freeway and 884 meters to the Southwest of the site is Lawndale Street. Around 622 meters far from the site, there are railways connecting industries in the area. Close to a residential area, it is believed that the pollution from industries could affect citizen health in the area [3]. Moreover, the site is close to Texas Medical Center, the

largest medical center in the world where hosts several cancer patients from different parts of US. Next to the trailer at the site, a mobile station was operated by University of Houston (UH). This mobile station included a PTRMS to provide real-time monitoring data of selected VOCs as shown in Table 1. Close to the site, locations of two Continuous Ambient Monitoring Station, CAMs 1029 and CAMs 304, were shown in Figure 1 as ambient data were also collected from these two stations operated by Texas Commission on Environmental Quality (TCEQ).

2. Exposure description

The exposure system was installed inside a secure temperature controlled portable field laboratory building. The setup of this exposure system is shown in Figure 2. At least 4 hours before exposure, the exposure chamber was cleaned with ethanol and the complete system powered including the heated incubator, humidifier, and the peristaltic water (Fisher HPLC grade) pump. This protocol allows the humidifier to be wetted and stabilize at a temperature of 37ºC, Relative Humidity of 60- 90%, and 5% CO_2.

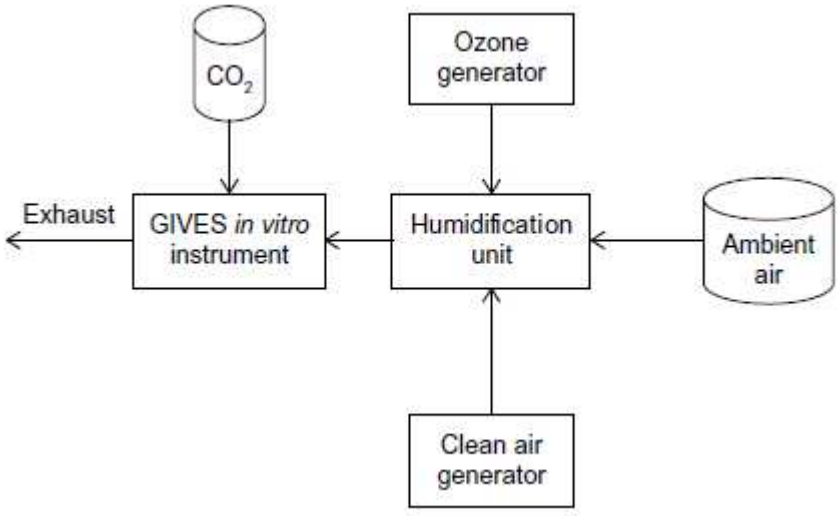

Figure 2: Schematic of the sample airflow from sources (clean air, ozone, and ambient air) through the humidification unit and into the GIVES exposure instrument housing the lung cells.

The epithelial lung cell A549 was used in the field deployment because of its commercial availability, robustness and worldwide reproducibility with consistent results. A549 cells from UNC lab were frozen and transported to the cell culture lab in Baylor Medicine College, Houston, Texas. As they arrived, cells were thawed and moved to a T75 flask with 20 ml of 37^{o}C culture media (F-12K, fetal bovine serum (FBS), Penicillin/Streptomycin). The cells were cultured using air-liquid interface system in which the basolateral surfaces of cells were cultured by media and the apical surfaces of cells without media were contacted directly with air pollutants. Until confluence (80%), cells were split to a new T75 flask of 1,800K of cell/ml and placed to each well 800ml of 850k cells/ml to 9-12 wells plate (Corning) overnight prepared for the next day morning exposure.

To expose cells, the culture media was replaced with starving media (F-12K, BSA, Penicillin/Streptomycin) for four hours. Prior to exposure, the starving media was removed and new starving media added. Plated cells were then placed in an insulated blackout box and transported to the field site. During transport, a level was maintained using a "bubble" balance. Once placed in the instrument the cells were maintained in an environment of 5% CO_2 and 37^{o}C and 56-90% relative humidity. The first experiments in the field were three exposures to clean air (negative control) and two exposures to 400 ppb Ozone (positive control). In addition, cells were placed in an incubator as a control. For these field experiments, flowrates of 1L/min were checked using a Gilibrator flow meter before connecting to a medical clean air source or an ozone generator. The ozone generator was sampled with a calibrated ozone meter (Monitor Labs 9811). After exposures, the cells were covered and place in the insulated blackout box and transported immediately back to the Baylor Medical School laboratory where a UNC team member received the package to deliver the cells to the laboratory incubator for expression during the night.

For the ambient exposures, the in vitro exposure instrument sample inlet was connected to a Teflon (FEP, ¼ inch O.D) sample tube mounted on a 7 feet long mast on top of the field laboratory. The end of the ambient sample line at the top of the mast was protected with a funnel to prevent rain droplets and insects from touching the tube inlet. All ambient exposures started at approximately 12 PM and lasted 4 hours. We conducted 5 days of ambient air exposure, February 18, 29, 24, 25, and 26 of 2015. After 16 hours, post-exposure cells were collected with RNA in Trizol and supernatant separately. Then samples were stored in a box with dry ice and transport by car to UNC lab. Cell exposure samples were frozen at -20^{o}C during the field campaign and then driven back to UNC on dry ice (-80^{o}C) during a 48-hour driving time.

3. Supernatant Analysis

Upon arriving in UNC lab, cells were frozen at a temperature of -80^{o}C. Then supernatant from samples was thawed and measured for the cytotoxicity. For the cytotoxicity, supernatant samples were dyed and analyzed for the enzyme lactase dehydrogenase (LDH) using Pierce™ LDH Cytotoxicity Assay Kit (Life Technologies) [14]. Incubator control and exposed sample wells were also analyzed for LDH cytotoxicity in triplicate and scanned absorbance reading for 492nm and 690nm wavelength using Thermo / LabSystems 352 Multiskan MS Microplate Reader. Applying the Grubb's test, the reading outliers were indicated as those with less than 5% probability of occurring relative to a normal distribution [15]. Fold increase of LDH level was calculated by dividing the mean levels of exposed samples by those of control samples after subtracting them with BSA blank (starving media). Data for LDH are presented as the mean ± standard error from the mean and expressed as fold increase over control. Data were analyzed using an unpaired Student's t-test where differences were considered significant if $p \leq 0.05$. Cells

were first exposed to clean air as a negative control. This allowed us to investigate any potential problems with cell culture media evaporation that could lead to cell desiccation or to contamination of any type including carry-over from previous exposures. No statistical difference in LDH levels between incubator controls and clean air exposures were observed.

4. RNA extraction

Following a revised Qiagen miRNeasy protocol, total RNA was extracted from exposed and control samples. In short, samples stored in Trizol were thawed at room temperature (25ºC) and were homogenized using a QIAshredder. Then the homogenized cell constituents were incubated at 25ºC for 5 minutes. After 5 minutes, 200µl chloroform was added to the mixture, incubated at 25ºC for 3 minutes, then centrifuged for 15 minutes with the rate of 12,000 x g at 4°C. The aqueous phase of the mixture was transferred to a new tube containing 750 µl of biological grade ethanol and mixed thoroughly. This new mixture was then spun using a Qiagen miRNeasy spin column and the end of extraction were followed the manufacturer's instructions.

5. NanoString Gene Expression Assays

From each extracted sample, 50ng of RNA was checked for biological qualification and evaluated for comparative expressions of various gene targets using NanoString's nCounter Inflammation and PanCancer Panels of the NanoString technology [16]. The NanoString technology employed a specific binding of a unique molecular barcode for each targeted mRNA and a molecular imaging for direct hybridization. Probes for each target included a visible reporter probe and a biotinylated capture probe, hybridizing to target mRNA overnight at 65°C. After the excess and non-targeted probes were removed, the remaining probes were immobilized on a

streptavidin-treated cartridge. The molecules were fixed using an electrical field and the cartridge was moved to the nCounter instrument where epifluorescence microscopy and a CCD camera were employed to capture images of target-probe complexes. The molecular images were processed within the nCounter instrument and counts were tabulated and reported in a .csv format.

6. NanoString Normalization and Analysis

NanoString data were processed separately using two software, SAS (SAS Institute, Cary, NC) and Partek Genomic Suite (St. Louis, MO). Data were normalized in a two-step process following the manufacturer's guide. First, positive control normalization was carried out. Second, housekeeping gene normalization was performed for each panel. Both normalization processes provide quality control for batch effect and artifact error. Differential expression was identified as the condition of a $p<0.05$, and a fold change $>|1.5|$ was met [9]. False discovery rates (FDR) corrected p values and fold changes were also calculated and reported.

CHAPTER 3: RESULTS

1. Ambient Measurements

We collected meteorological data and ambient measurements from two Continuous Ambient Monitoring Station (CAMS) operated by the Texas Commission on Environmental Quality (TCEQ). The two CAMS sites were CAMS 1029 and CAMS 403. Both sites report hourly average data [17]. CAMS 1029 was located at 29° 43' 7.00" North and 95° 15' 35.00" West and was 0.4 miles to the west of the study site at 9700 Manchester Street, Houston, Texas. CAMS 1029 recorded pollutant as total non-methane organic compounds and meteorological data as wind speed, wind direction, maximum wind gust, and outdoor temperature. All data from CAMS 1029 is reported in Table 2. CAMS 403 was situated at 29° 44' 1.00" North and 95° 15' 27.00" West which was around 1 mile to the North of the study site. CAMS 403 recorded meteorological data as wind speed, wind direction, maximum wind gust, outdoor temperature, dew point temperature, relative humidity, solar radiation, ultraviolet radiation barometric pressure, precipitation, some pollutants as carbon monoxide, sulfur dioxide, nitric oxide, nitrogen dioxide, oxide of nitrogen, ozone, $PM_{2.5}$ and GC data as ethane, ethylene, propane, benzene, etc. Concentrations of the pollutants were presented in Table 1. Dewpoint temperature, relative humidity, solar radiation, ultraviolet radiation barometric pressure, precipitation from this station were also included in Table 2.

Moreover, we collected VOC data measured by researchers from the University of Houston mobile lab. The mobile lab operated a PTR-MS and measured methanol, acetonitrile,

acetaldehyde, acetone, benzene, toluene, C2 benzenes, C3 benzenes, Styrene, and MEK. The pollutant concentrations were recorded three to five data points every second. These data were averaged and reported into hourly values.

As shown in Table 1, the in vitro ambient exposures days showed differentiated composition when compared to the clean air. The biology was exposed to peaks of hourly ozone concentrations of 38 ppb and NOx at 29.5 ppb. There were also exposures to a variety of VOCs, most importantly aromatics where toluene peaked at 4.98 ppb, all benzene at 15 ppb, and xylene at 1.44 ppb. Table 2 provides some meteorological parameters from the exposure period. There was no precipitation on these days and resultant wind speed suggests calm winds, approximately 3 mph, indicating that for a 4-hour exposure period, cells were exposed to air pollution sources up to 12 miles away if winds were consistent in direction.

Table 1. Ambient measurements from CAMs monitors and the University of Houston PTR-MS (UH) of selected pollutants that were exposed to the biology. For all data, the average, median, min, max values were calculated using hourly average data from 12-4 pm on February 8, 24, and 26

Species	Average	Median	Min	Max	Site
Ozone [b]	26.67	28.00	12.00	38.00	CAMs
Total Non-Methane Organic Compounds [a]	18.69	20.16	8.20	27.03	CAMs
Oxides of Nitrogen [b]	14.87	16.4	4.2	29.5	CAMs
Ethane [a]	17.36	17.44	12.52	24.86	CAMs
Propane [a]	13.65	12.84	10.47	17.61	CAMs
Methanol [a]	2.35	2.29	1.36	3.66	UH
n-Butane [a]	8.61	8.76	6.36	10.36	CAMs
Acetone [a]	5.15	4.95	2.68	7.36	UH
Acetaldehyde [a]	1.65	1.62	0.94	2.28	UH
Isobutane [a]	3.29	3.28	2.80	3.92	CAMs
Isopentane [a]	3.71	3.75	2.75	4.90	CAMs
Ethylene [a]	1.38	1.16	0.74	2.32	CAMs
n-Pentane [a]	2.74	2.65	2.30	3.20	CAMs
Acetylene [a]	1.01	1.06	0.64	1.32	CAMs
Toluene [a]	2.71	2.87	0.06	4.98	UH
C2_Benzenes [a]	3.04	3.00	0.59	6.16	UH
Benzene [a]	2.11	2.16	0.61	3.72	UH
MEK [a]	1.37	1.16	0.85	2.53	UH
C3_Benzenes [a]	2.81	2.19	0.36	8.19	UH
Carbon Monoxide [c]	0.24	0.20	0.20	0.30	CAMs
Sulfur Dioxide [b]	0.21	0.20	-0.10	0.60	CAMs
Propylene [a]	0.58	0.63	0.33	0.81	CAMs
n-Hexane [a]	1.05	1.02	0.84	1.38	CAMs
Acetonitrile [a]	0.28	0.32	-0.03	0.49	UH
p-Xylene + m-Xylene [a]	0.82	0.72	0.56	1.44	CAMs
Styrene [a]	0.43	0.61	-0.71	1.21	UH
[a] ppbC, [b] ppbV, [c] ppm					

Table 2. Meteorological measurements for exposed biology

Parameters	Average	Median	Minimum	Maximum
Resultant Wind Speed (MPH)	2.99	-	-	-
Outdoor Temperature (degree F)	50.3	52.8	38.1	61.8
Maximum Wind Gust (MPH)	12.3	13	8.6	16.8
Dew Point Temperature (degree F)	31.4	32.5	27.2	34.3
Relative Humidity (%)	52.6	49.3	27.2	78.4
Precipitation (inches)	0	0	0	0

2. Cytotoxicity

Figure 3 shows the LDH results from the analysis of the supernatant. The LDH assay was used to quantify the cytotoxicity of the three negative control experiments (clean air exposures) which were reported as fold change over the incubator control (lung cell housed in the incubator). All data were then normalized to a maximum cytotoxicity of a 7.5-fold change [18]. As shown in the figure the clean air exposures did not have a significant increase in LDH over the control. The ozone exposures show a 3.5-4-fold increase over control. Both the clean air and ozone exposures replicated responses seen in the laboratory. This is significant as it means that the instrument itself and the field deployment of the biology did not have an adverse impact on the cells. Thus, the toxicity responses from the ambient exposures are solely from the sampled gases.

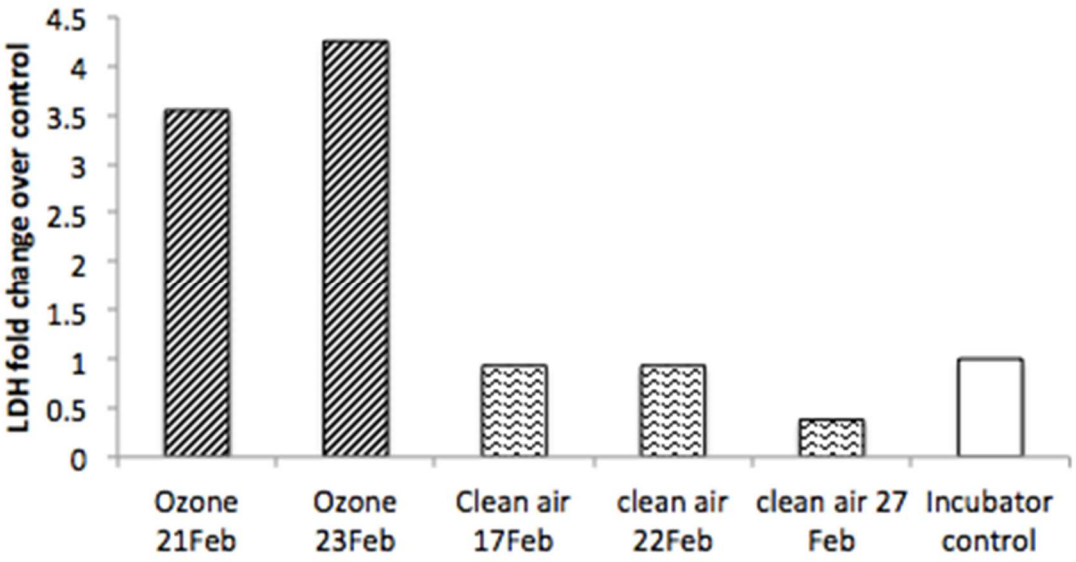

Figure 3. LDH data from the supernatant analysis

3. Genomic Expression Response

To determine whether ozone, ambient exposure and/or clean air alter the mRNA expression level of inflammation and cancer-related genes, field experiments and laboratory exposures were carried out in A549 cells. Of the 594 immune-related genes and 730 pan cancer related genes that were measured, 11 genes showed significant ($p<0.05$) differential expression in response to one of the treatments. The detail of expressed genes was shown in Table 3. In the heat map in Figure 4, red color represents a positive value of gene expression while blue color represents a negative value of gene expression. The positive value of gene expression represents an upregulated gene changed due to exposure while the negative value of gene expression represents a downregulated gene expressed because of exposure.

Table 3. Differentially Expressed Genes in response to air-toxics exposures

Gene Name	Full Gene Name	Biological Function
ALOX5	Arachidonate 5-Lipoxygenase	Important mediator inflammatory and allergic conditions. Mutations in the promoter region lead to a diminished response to anti-leukotriene drugs used in the treatment of asthma and may also be associated with atherosclerosis and several cancers.
C2	Complement Component 2	Deficiency in *C2* reported to be associated with certain autoimmune diseases. SNPs in this gene have been associated with altered susceptibility to age-related macular degeneration.
CCL11	Chemokine (C-C Motif) Ligand 11	An antimicrobial chemokine, from a superfamily of secreted proteins involved in immunoregulatory and inflammatory processes. CCL11 has chemotactic activity for eosinophils, but not mononuclear cells or neutrophils. It is also involved in eosinophilic inflammatory diseases such as atopic dermatitis, allergic rhinitis, asthma and parasitic infections
CCL24	Chemokine (C-C Motif) Ligand 24	A cytokine, a family of secreted proteins involved in immunoregulatory and inflammatory processes. The CCL24 protein has a chemotactic activity on resting T lymphocytes, a minimal activity on neutrophils, and is negative on monocytes and activated T lymphocytes. It is also a strong suppressor of colony formation by a multipotential hematopoietic progenitor cell line.
DEFA1	Defensin, Alpha 1	Defensins are a family of proteins involved in host defense. They are abundant in the granules of neutrophils and also found in the epithelia of mucosal surfaces including the respiratory tract. The protein encodedDEFA1 is found in neutrophils and likely plays a role in phagocyte-mediated host defense.
FGFR3	Fibroblast Growth Factor Receptor 3	The family of fibroblast growth factor receptor (FGFR) family, is a transmembrane protein. The extracellular portion of the protein interacts with fibroblast growth factors, resulting in a signal cascade, and influencing mitogenesis and differentiation. FGFR3 plays a role in bone development and maintenance. Mutations in this gene lead to craniosynostosis and multiple types of skeletal dysplasia.
IFIT3	Interferon-Induced Protein with Tetratricopeptide Repeats 3	IFIT3 is a novel antiviral gene, the protein that it codes for is an extracellular protein. Member of these gene families are potent antiviral effectors that function to suppress the entry of a broad range of enveloped viruses and modulate cellular tropism independent of viral receptor expression.
IL11	Interleukin 11	The protein encoded by this gene is a part of a cytokine family that drive the assembly of multi subunit receptor complexes involved in transmembrane signaling receptor. This cytokine is shown to stimulate the T-cell-dependent development of immunoglobulin-producing B cells. It is also found to support the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells.

IL12B	Interleukin 12B	This gene encodes a subunit of interleukin 12, a cytokine that primarily acts on T and natural killer cells, and serve as an essential inducer of Th1 cells development. This cytokine has been found to be important for sustaining a sufficient number of memory/effector Th1 cells to mediate long-term protection to an intracellular pathogen. Overexpression of this gene was observed in the central nervous system of patients with multiple sclerosis (MS), suggesting a role of this cytokine in the pathogenesis of the disease. The promoter polymorphism of this gene has been reported to be associated with the severity of atopic and non-atopic asthma in children.
MX2	MX Dynamin-Like GTPase 2	The protein encoded by this gene has a nuclear and a cytoplasmic form. The nuclear form is localized in a granular pattern in the heterochromatin region beneath the nuclear envelope. This protein is upregulated by interferon-alpha but does not contain the antiviral activity of a similar mycovirus resistance protein 1.
PTGIR	Prostaglandin I2 (Prostacyclin) Receptor	The protein encoded by this gene is a member of the G-protein coupled receptor family 1 and has been shown to be a receptor for prostacyclin. Prostacyclin, the major product of cyclooxygenase in macrovascular endothelium, elicits a potent vasodilation and inhibition of platelet aggregation through binding to this receptor.

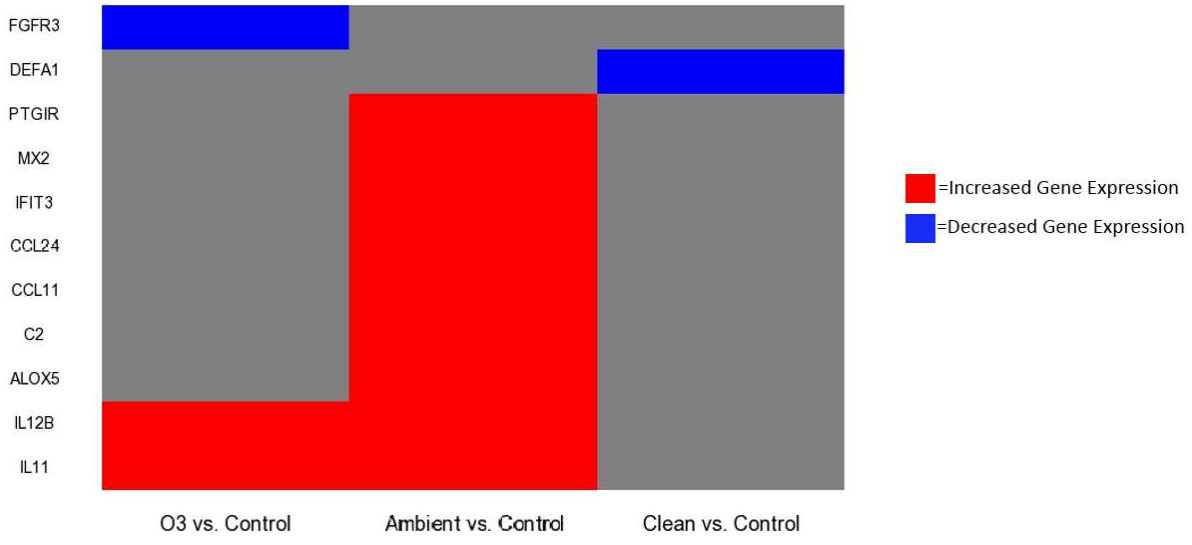

Figure 4. Heat map displaying the 11 differentially expressed genes in association with the exposures

Of the 11 genes that showed significant changes in gene expression, 9 genes were associated with ambient air exposures, 3 were expressed due to ozone exposures and only 1 was altered because of clean air exposure. The 9 genes changed in response to ambient air were associated with immune and inflammatory response: Arachidonate 5-Lipoxygenase (*ALOX5*), Complement Component 2 (C2), Chemokine (C-C Motif) Ligand 11(*CCL11*), Chemokine (C-C Motif) Ligand 24(*CCL24*), Defensin, Alpha 1(*DEFA1*), Interferon-induced protein with tetratricopeptide repeats 3(*IFIT3*), Interleukin 11(*IL11*), Interleukin 12B(*IL12B*), MX Dynamin-Like GTPase 2(*MX2*), and Prostaglandin I2 (Prostacyclin) Receptor (*PTGIR*). The final gene, Fibroblast Growth Factor Receptor 3 (*FGFR3*), is involved in mitogenesis and differentiation specific to bone development and maintenance.

CHAPTER 4: DISCUSSION

The Benzene and other Toxics Exposure (BEE-TEX) campaign was a field deployment aimed at measuring exposure source attribution of air toxics. The study was conducted in 2015 in the Houston Ship Channel where intense industrial sources of air toxics are located in close proximity to residential neighborhoods. During BEE-TEX, we were able to successfully deploy for the first time in the field our novel in vitro instrument [11]. Using this instrument, we exposed A549 epithelial lung cells to 5 ambient days. In addition to these days, we also conducted 3 days of clean air exposure (negative control) and 2 days of 0.4 ppm ozone exposure (positive control). After all exposures, the supernatant was collected and the total RNA from the biology. This included the small RNAs ≥18 nucleotides. The supernatant material was used to quantify LDH proteins. The RNA extracted from A549 cells was evaluated for comparative expression of various gene targets using NanoString's nCounter Inflammation and PanCancer Panels.

These biomarkers were first analyzed to ensure the successful deployment of the instrument. Both the results from the clean air and ozone exposures were consistent with similar exposures conducted in the laboratory. This is significant as it means that the instrument itself and the field deployment of the biology did not have an adverse impact on the cells. Thus, the toxicity responses from the ambient exposures are solely from the sampled gases.

The ambient exposures days showed differentiated composition when compared to the clean air. The biology was exposed to peaks of hourly ozone concentrations of 38 ppb and NOx at 29.5 ppb. There were also exposures to a variety of VOCs, most importantly aromatics where toluene peaked at 5 ppb, benzene 15 ppb, and xylene at 1.44 ppb. There was no precipitation on these days and resultant wind speed suggests calm winds. For the ambient days, the exposed biology mRNA was used to determine the levels of expression of inflammation and cancer-related genes. Of the 594 immune-related genes and 730 pan cancer-related genes that were measured, 11 genes showed significant ($p<0.05$) differential expression in response to one of the treatments. Of the 11 genes that showed significant changes in gene expression, 10 ones were associated with immune and inflammatory response: Arachidonate 5-Lipoxygenase (ALOX5), Complement Component 2 (C2), Chemokine (C-C Motif) Ligand 11(CCL11), Chemokine (C-C Motif) Ligand 24(CCL24), Defensin, Alpha 1(DEFA1), Interferon-induced protein with tetratricopeptide repeats 3(IFIT3), Interleukin 11(IL11), Interleukin 12B(IL12B), MX Dynamin-Like GTPase 2(MX2), and Prostaglandin I2 (Prostacyclin) Receptor (PTGIR). The final gene, Fibroblast Growth Factor Receptor 3 (FGFR3), is involved in mitogenesis and differentiation of bone development and maintenance.

In this study, the success of this first field deployment for human lung cell exposure to ambient air was highlighted. Moreover, the limitations of the study were acknowledged. The gene expressions were analyzed as a group, while concentrations of a specific chemical would impact the specific gene expression significantly. Another limitation was the limited time and location of the study. Due to limited exposure of 4-hour during the afternoon, the exposures during the night, when the atmospheric transformations are minimal, were not captured and compared. The longer period of time would be benefited for more accurate observations. If the study could be extended

to other cities or rural areas, we would have more knowledge of the biological expressions due to exposures to different urban and background gaseous mixtures. Finally, we only analyzed RNA of the selected sets of genes related to inflammation and cancer pathways. Other genes related to other pathways and other biological effects to DNA, proteins could also be considered to understand more about the effects of mixtures of gases in the atmosphere.

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