

STYRENE EXPOSURE AND NEUROLOGIC EFFECTS IN RESIDENTS OF THE US  
GULF STATES

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## ABSTRACT

Emily Jean Werder: Styrene exposure and neurologic effects in residents of the US Gulf States  
(Under the direction of Lawrence S. Engel)

Styrene is an established neurotoxicant at occupational levels, but epidemiologic studies to date have focused on highly exposed workers. We examine whether neurologic effects are associated with styrene at environmental levels, and attempt to identify underlying sources of uniquely elevated exposure levels among Gulf coast residents.

In Aims 1 and 2, we utilized data from the Gulf Long-term Follow-up Study and the nested Chemical Biomonitoring Study (CBS) to assess predictors of blood styrene levels (N = 667). In Aim 3, we estimated cross-sectional associations between ambient styrene exposure and neurologic symptoms (N = 21,962), as well as peripheral neurologic function (N = 2,956). Among CBS participants, we assessed blood styrene in relation to neurologic symptoms (N = 874) and peripheral neurologic function (N = 310). Ambient exposures were modeled as quartiles, and blood exposures were divided at the median or 90<sup>th</sup> percentile. We estimated prevalence ratios using log-binomial regression, and differences in continuous outcomes using linear regression.

Blood styrene levels are 2-3 times higher in CBS compared to the U.S. general population. Smoking, housing characteristics, and recent behaviors were predictors of blood styrene levels. Neither ambient styrene concentrations nor industrial styrene

emissions were determinants of blood styrene levels. The highest quartile of ambient styrene was associated with increased central (PR = 1.20, 95% CI: 1.09, 1.32) and peripheral (PR = 1.12, 95% CI: 1.02, 1.23) nervous system symptoms, as well as impairments in vision (mean difference = -0.15, 95% CI: -0.25, -0.04), vestibular ( $\beta$  = -4.65 mm/s, 95% CI: -8.20, -1.10), and sensory function ( $\beta$  = -0.12 log microns, 95% CI: -0.22, -0.01). We observed statistically significant monotonic exposure-response relationships between ambient styrene concentration and many neurologic endpoints. The relationship was less clear for blood styrene exposure, with some suggestive effects.

Personal predictors of increasing blood styrene levels were largely consistent with previous literature. Our measures of increased regional exposure opportunity do not fully explain these elevated blood styrene levels. Increasing ambient styrene exposures elicited consistent neurotoxic effects, as well as some notable associations with measured blood styrene. Environmental styrene exposure levels may be sufficient to elicit subclinical neurotoxic effects.

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## LIST OF ABBREVIATIONS

2,5-DMF	2,5-Dimethylfuran
AL	Alabama
AMA	Ambient Monitoring Archive
ATSDR	Agency for Toxic Substances and Disease Registry
BTEX	Benzene, Toluene, Ethylbenzene, and Xylenes
CATI	Computer Assisted Telephone Interview
CBS	Chemical Biomonitoring Study
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CNS	Central Nervous System
DWH	<i>Deepwater Horizon</i>
EPA	United States Environmental Protection Agency
FL	Florida
GuLF STUDY	Gulf Long-term Follow-up Study
HAP	Hazardous Air Pollutant
LA	Louisiana
LOD	Limit of detection
MS	Mississippi
NATA	National Air Toxics Assessment
NEI	National Emissions Inventory
NHANES	National Health and Nutrition Examination Survey
PNS	Peripheral Nervous System
PR	Prevalence Ratio

PSL	Point Source Location
TX	Texas
US	United States
VOC	Volatile Organic Compounds

## CHAPTER I: INTRODUCTION & SPECIFIC AIMS

### A. Introduction

Styrene is an industrial hydrocarbon used in plastics, fiberglass, rubber, and resins to manufacture consumer products and commercial and residential building materials. Manufactured styrene products include insulation, fiberglass boats, automotive parts, car tires, Styrofoam, and plastic drinking glasses [1]. Exposure to styrene in the general population occurs primarily through inhalation of tobacco smoke, off-gassing of building materials, and vehicle and industrial emissions [2, 3]. The Gulf States are home to many styrene emitting industries and over half of all U.S. styrene production [1, 4]. Styrene is an established neurotoxicant at occupational levels [1, 5, 6], but has not been studied at environmental levels experienced by the general population. Epidemiologic studies to date have focused on highly exposed workers, whose average blood levels were 25 times higher than those of the general population [7-14].

We conducted a study of environmental styrene exposure and self-reported neurologic symptoms by taking advantage of data that were collected in the ongoing Gulf Long-Term Follow-up Study (GuLF STUDY). The study included individuals who participated in the 2010 *Deepwater Horizon* (DWH) oil spill response and cleanup and comparison subjects who did not. Neurologic symptoms were ascertained for the entire cohort (N=32,608) at the enrollment interview, which occurred between 2011 and 2013. Approximately two to three years after the oil spill (May 2012-July 2013), a subset of

participants (N=994) living in the Gulf region provided blood specimen for measurement of volatile organic compounds (VOCs), including styrene, as part of a Chemical Biomonitoring Study (CBS). Blood styrene levels collected from CBS participants are two to three times higher than those reported in the National Health and Nutrition Examination Survey (NHANES) [15], but considerably lower than levels typically observed among occupationally exposed populations. Because styrene is rapidly cleared from the body and blood measurements were obtained two to three years after the oil spill, these levels represent usual, ongoing exposures (i.e., they are not due to oil spill cleanup work). Although the sources for styrene levels of this magnitude are not well understood, increased exposure opportunity from industrial emissions in the Gulf region is plausible.

In Specific Aim 1, we evaluate individual characteristics and estimate ambient styrene concentration as predictors of measured blood styrene levels among CBS participants. Individual covariate information is obtained from GuLF STUDY interview/questionnaire data. Ambient styrene estimates, from the 2011 National Air Toxics Assessment (NATA) [16], are used to evaluate the contribution of ambient styrene, in addition to exposure due to individual-level demographic, lifestyle, and housing characteristics, to variability in blood levels.

In Specific Aim 2, we evaluate spatial patterns among point sources as predictors of blood styrene levels among CBS participants. Potential point sources of styrene emissions were identified using the United States Environmental Protection Agency's (EPA) National Emissions Inventory (NEI) from 2011 [17]. Exposure is assigned based on proximity, density, and characteristics of NEI styrene point sources near the



participant's residence. These distance-based metrics of exposure address diffusion of styrene emissions over space, allowing for exposure variability within and across census tract boundaries.

In Specific Aim 3, we assess the exposure-outcome relationships between both metrics of styrene exposure (estimated ambient concentrations and measured blood levels) and indicators of neurologic function (self-reported symptoms and quantitative neurologic function tests) in the full cohort and the CBS. The 2011 NATA annual average ambient styrene concentration corresponding to an individual's home census tract is applied as estimated styrene exposure for all cohort members residing in the Gulf region, and measured blood styrene are used for separate analyses among the CBS participants. We evaluate cross-sectional associations between ambient styrene exposure and self-reported neurologic symptoms among all study participants residing in the Gulf region (N=26,828), and also using blood styrene in the CBS subset. Among the 3,400 individuals who completed a follow-up clinical examination in 2014-2016, which included detailed neurological testing, we explore associations between styrene exposure and performance on neurologic tests.

The purpose of this study is to examine whether neurologic effects are associated with styrene at levels relevant to the general population, accounting for other risk factors, and to identify underlying sources of these exposure levels. Specific Aims 1 and 2 support the larger purpose of estimating health effects of styrene by evaluating different metrics of styrene exposure and elucidating potential confounding factors in the exposure-outcome relationship (Figure 1).

## **B. Specific Aims**

**Specific Aim 1: Analyze individual covariate information, as well as modeled environmental styrene concentrations, as determinants of blood styrene levels.**

Candidate exposure opportunities were derived from questionnaires obtaining detailed exposure information about VOCs with emphasis on the previous 24 hours. Additionally, NATA 2011 estimated annual average ambient census tract styrene concentrations were applied as a surrogate of usual environmental styrene exposure. Linear regression models are used to evaluate predictors of blood levels.

*Hypothesis 1:* Usual lifestyle factors and exposure opportunities account for more variability in measured blood styrene levels than do ambient styrene concentrations.

*Hypothesis 2:* NATA 2011 annual styrene concentrations correlate with blood styrene.

**Specific Aim 2: Spatially evaluate point sources of styrene emissions as predictors of blood styrene levels.**

Facilities emitting airborne styrene are ascertained from the 2011 NEI. Proximity, density, and intensity of facilities are analyzed to quantify spatial gradients between ambient point source emissions and measured blood levels. This approach accounts for spatial variability that is smoothed over in NATA estimates. Point source characteristics are also evaluated as predictors of blood styrene. The variability in blood styrene levels explained by point sources is estimated using least squares linear regression.

*Hypothesis:* Measured blood styrene levels vary as a function of residential proximity to and intensity of, point source styrene emissions.

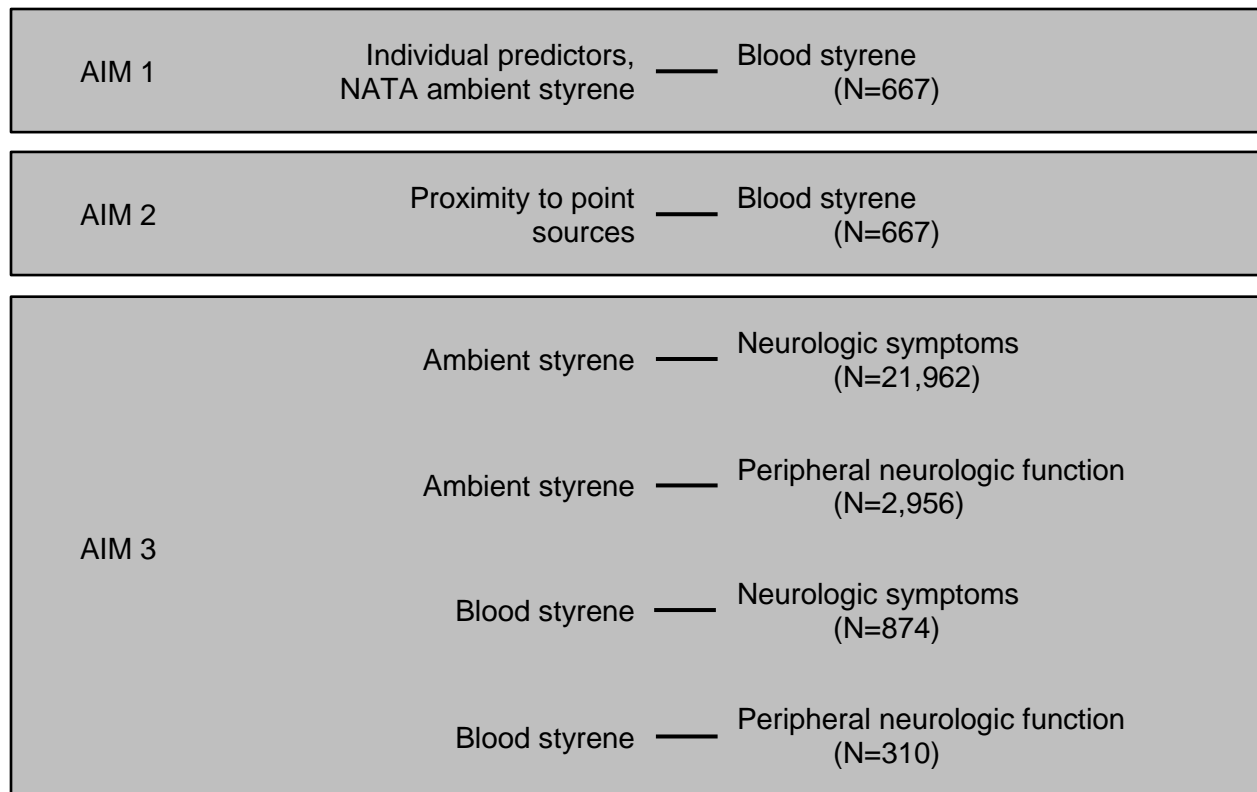
**Specific Aim 3: Estimate associations between measured and modeled styrene exposure and indicators of neurologic function.**

Associations with neurologic outcomes are estimated for ambient and blood styrene among CBS participants. Neurologic symptoms were ascertained at enrollment, and a subset of participants completed follow-up peripheral neurologic function testing between 2014 and 2016. Associations between styrene exposure and neurologic effects are estimated using multivariate linear and log-binomial regression.

*Hypothesis 1:* Styrene exposure is associated with symptoms of neurologic impairment.

*Hypothesis 2:* Peripheral neurologic function is inversely associated with styrene exposure.

Figure 1. Organization of specific aims.



### **C. Innovation**

**This study is the first to investigate the neurotoxicity of styrene at levels relevant to the general population.** Previous studies have examined health effects of styrene in exposed worker populations whose average blood levels were 25 times higher than those of the general population, limiting extrapolation to the health effects from typical environmental exposures. Participants in the proposed study have average blood styrene levels two to three times higher than those reported in NHANES. These levels are considerably lower than occupational exposures, but higher than typical environmental exposures experienced in the U.S. population. This range provides an excellent opportunity to detect any exposure-response relationships at lower exposure ranges. Thus, the present study is the first to determine whether neurologic effects observed among highly exposed occupational populations are also observed for styrene exposure more typical of the general population. Moreover, blood styrene levels were measured two to three years after the oil spill and, therefore represent contemporary, not oil spill cleanup-related exposures. These measurements show virtually no correlation with cleanup worker status. The exposure profile of these study participants is ideally suited to investigate neurologic symptoms in relation to levels of styrene exposure relevant to the general population.

**This study investigates an understudied population.** Few studies have investigated this socioeconomically disadvantaged, medically underserved, and racially diverse population. This study, together with the parent GuLF STUDY, can provide important insights into the general health issues faced by this population.

## CHAPTER II: BACKGROUND AND SIGNIFICANCE

### A. Styrene exposure

#### Sources of styrene

Styrene, an industrial hydrocarbon derived from ethylbenzene, is widely used in plastics, fiberglass, rubber, and resins to manufacture consumer products and commercial and residential building materials. When styrene monomer, a colorless liquid, is synthesized into polystyrene polymer, it becomes a rigid plastic at room temperature [3]. After the manufacture, use, and disposal of styrene-based products, styrene is released primarily into air, though smaller amounts are detected in soil and water [18]. Ambient styrene, a volatile organic compound, breaks down in the atmosphere within 1 to 2 days [1].

Styrene production in the U.S. has been steadily increasing since its initiation in 1938, and the U.S. now produces over 12 billion pounds of styrene annually, with atmospheric emissions of 28 million pounds per year [1, 19]. The main sources of styrene emissions to air include motor vehicle exhaust (approximately 30%), composites and boat building (approximately 40%), and all other sources (approximately 30%) [20].

The largest U.S. styrene production facilities, accounting for more than half of all production, are located in Louisiana and eastern Texas [3]. In addition to a prolific

petrochemical industry, the Gulf region is home to many industrial and manufacturing facilities that use and emit styrene in the production of plastics, rubber, and fiberglass. Motivated by economic efficiency, this geographic clustering of industries relying on a common set of resources potentially exposes Gulf residents to a disproportionately high intensity of environmental styrene emissions from petrochemical, manufacturing, and coastal fishing and boating operations [21].

Styrene is used predominantly in the production of polystyrene plastics and resins, and to a lesser extent, as an intermediate in the copolymers styrene-acrylonitrile, acrylonitrile-butadiene-styrene, styrene-butadiene rubber, and styrene-butadiene latex [3]. These plastics, resins, and copolymers are commonly used in building materials and a variety of consumer products, including: fiberglass boat hulls, tubs, and shower stalls; packaging materials, paper coatings, food storage containers, and Styrofoam; industrial hoses, plastic piping, electrical insulation and wiring, building insulation, refrigerator liners, carpet backing, and latex paints; housewares and appliances; automotive parts, car tires, and car battery enclosures; toner found in photocopiers and printers [1, 4, 22]. These products mainly contain polystyrene chains, as well as a small amount of unlinked styrene. Smaller amounts of styrene are used for less common thermoplastics, laboratory and water purification uses (ion-exchange resins), and glues and adhesives [20].

Styrene is typically disposed of through absorption on vermiculite, incineration, or containment in EPA-permitted landfills [1]. Groundwater and soil styrene levels indicate leaching from landfills, and monitoring data document elevated ambient styrene closer

to landfills [6, 23]. Exposure to styrene from hazardous waste sites is potentially important, but the magnitude of the problem is unknown [6].

### **Routes of human exposure**

Human exposure to styrene can occur through inhalation, ingestion, or dermal contact. Inhalation of airborne styrene is the primary route of exposure, and the main source of concern for health effects due to styrene toxicity [24]. Ingestion and dermal exposure to styrene are possible, though inhalation accounts for over 90% of styrene exposure in the general population [6, 25].

Air styrene is released from industries using or manufacturing styrene, automobile exhaust, cigarette smoke, and photocopiers and printers. Styrene is commonly detected in urban air, near industrial sites and landfills, and in high traffic areas, although typically at levels substantially lower than in occupational settings. Rural or suburban air generally contains lower concentrations of styrene than urban air [22]. National-scale ambient styrene monitoring is limited, with poor spatial and temporal coverage, but local-scale studies have demonstrated that residents living near high-emitting styrene production or processing facilities frequently experience chronic elevated exposure from emissions [20, 26, 27] (i.e., 28 million pounds in 2013 [19]). While outdoor air styrene concentrations are driven by industrial and mobile emissions, indoor air styrene levels primarily result from tobacco smoke, off-gassing of building materials and consumer products, and emissions from photocopiers and laser printers [1].

Very low levels of styrene occur naturally in strawberries, coffee, cinnamon, oats, wheat, peanuts, wine, beef, eggs, cheese, beer, and some legumes [1, 20]. Styrene detected in other prepared foods is attributed to migration from polystyrene packaging and containers [28]. The amount of styrene migration to food and drink is variable, but migration is consistently dependent upon the fat content and storage temperature [29, 30]. Higher fat content and temperature are associated with increased migration. In an experimental study of polystyrene containers and solvents, foods, and beverages, maximum observed migration for beverages and foods was 0.025% of the container's total styrene [30]. Detected concentrations vary widely, with higher levels resulting from packaging than those due to naturally occurring styrene [31]. Styrene levels in bottled water increase over time, suggesting leaching from the containers [32].

Due to styrene's rapid biodegradation and volatility, surface and groundwater levels are generally below 1 µg/L [22]. Exposure levels observed in municipal drinking water supplies are considered negligible [18]. However, styrene has been detected at significant levels in the groundwater of hazardous waste sites [33, 34]. If used as a local water supply, this water would confer significant, potentially hazardous exposure to styrene. The magnitude of styrene exposure due to oral consumption, whether due to natural dietary sources or leaching from food packaging, is much lower than that of inhalation exposure. While ingested styrene is detectable, these trace levels are generally considered too low for toxicity [1, 2, 6, 25, 31].

A very small amount of styrene may enter the body when skin comes into contact with liquids containing styrene. Oxidative stress is involved in skin damage due to liquid styrene exposure[1]. The potential for exposure to liquid styrene through skin contact is



likely limited to occupational opportunities, but because of very low percutaneous absorption in humans, this exposure route does not contribute significantly to the body burden of styrene-exposed plastics workers [6, 35].

### **Occupational exposure**

The highest styrene exposures generally occur in the workplace, where styrene-exposed workers have blood levels that are 25 times higher on average than those in the general population [5, 7, 36]. Approximately 330,000 U.S. workers are occupationally exposed to styrene [37]. Occupational styrene exposure has been assessed extensively in blood, urine, personal air, ambient air, and using occupational histories and job exposure matrices. Measured biomarker levels consistently predict exposure classification according to job title and occupational styrene-related activities [7, 10, 38-47].

Occupational exposure to airborne styrene, resulting from industrial production and use of styrene and styrene-based polymers and copolymers, vehicle emissions, and combustion processes[4, 22], has been well-characterized among workers in boat building, plastics, rubber, and polystyrene manufacturing [10, 13, 14, 25, 48-50]. The highest potential exposure occurs in the reinforced-plastics industry, where workers may be exposed to high air concentrations and potentially have dermal exposure to liquid styrene or resins. Workers involved in styrene polymerization, rubber manufacturing, and styrene-polyester resin facilities and workers at photocopy centers may also be exposed to styrene. In typical work environments, air concentrations are currently below 10 ppm except in portions of the reinforced plastics industry, where levels of 20 ppm or more are common [20].

## **Environmental exposure**

In the U.S. general population, daily exposure to styrene in air is estimated to be 18–54 µg/person, compared to 0.2–1.2 µg attributable to styrene in food [1]. Smoking a single cigarette delivers as much as 6 µg styrene [51]. Indoor air usually contains higher levels (0.07–11.5 ppb) of styrene than outdoor air (0.06–4.6 ppb), and inhalation of contaminated indoor air is considered the principal route of styrene exposure for the general population.

## **Outdoor air**

Emissions from industrial activities and motor vehicle exhaust are the primary sources of styrene in outdoor air. Ambient measurements of styrene typically show airborne concentrations less than 1 part per billion by volume (ppb), although concentrations exceeding 5 ppb have been detected in urban areas [20, 22]. The commonly observed urban-rural styrene concentration gradient is attributed to increased motor vehicle emissions [52].

The EPA compiled passively monitored ambient concentration data from three study areas throughout the United States from 1989-91 and reported an overall mean concentration of 0.13 ppb (0.55 µg/m<sup>3</sup>) [53]. More recent ambient styrene levels, measured at 74 different EPA monitoring sites across the five Gulf States in 2011, returned an observed mean styrene concentration of 0.04 ppb (SD, 0.3 ppb) (mean, 0.15 µg/m<sup>3</sup>; SD, 1.3 µg/m<sup>3</sup>). Among the 2011 Gulf monitors, the maximum observed styrene concentration was 20.9 ppb. Concentrations exceeding 1 ppb were detected at 12 different monitoring locations in the Gulf region during this time. Across the Gulf

States, average EPA estimated annual styrene concentrations partitioned by source were: 0.018  $\mu\text{g}/\text{m}^3$  for point sources, 0.008  $\mu\text{g}/\text{m}^3$  for mobile sources, and 0.004  $\mu\text{g}/\text{m}^3$  for all other sources [16].

Individuals living near domestic manufacturing or processing facilities may experience increased styrene exposure from point source emissions, totaling 47.3 million pounds annually in the U.S. [1]. Elevated ambient styrene concentrations are observed near styrene emitting industries, with clear dose-response relationships among distance, emissions volume, and ambient concentration. The reinforced plastic industry emits the largest quantities of styrene to the ambient air due to the nature of the production process.

In 2011, point source industrial styrene emissions in the Gulf region totaled 3,275 tons, compared to 671 tons of mobile styrene emissions, and 223 tons due to other sources [54]. Atmospheric dispersion simulations indicate that residential proximity to styrene emitting industrial facilities confers up to 15 ppb of annual average styrene exposure [26]. The effective exposure distance depends on emissions volume, stack height, and meteorology characteristics, but elevated concentrations were detected up to 10 kilometers away [55]. This exposure estimate takes into account average styrene concentrations, residential occupancy period, daily hours spent in the home, daily hours spent outdoors at home, indoor air levels, and residence air exchange rate.

Ambient monitoring of VOCs in New Jersey detected comparable mean and maximum styrene levels (mean, 0.16  $\mu\text{g}/\text{m}^3$ ; maximum, 0.60  $\mu\text{g}/\text{m}^3$ ) at mobile and industrial source-dominated sites [56]. Observed styrene levels were significantly higher (mean, 0.60  $\mu\text{g}/\text{m}^3$ ; maximum, 0.98  $\mu\text{g}/\text{m}^3$ ) at the commercial source-dominated

site [56]. This finding may reflect the combined effects of mobile and commercial sources because the commercial site was located in a high-traffic area.

### **Indoor air**

Inhalation of contaminated air is the principal route of styrene exposure for the general population [1]. Typically, indoor air contains higher styrene levels than outdoor air [51]. Mean indoor air levels of styrene have been reported in the range of 0.1–50.0  $\mu\text{g}/\text{m}^3$  (0.02-11.7 ppb), and are attributed to emissions from building materials, consumer products, and tobacco smoke [24]. Specific materials off-gassing styrene include carpets, floor tiles, insulation, office copiers, laser printers, disinfectants, plastics and fiberglass, paints, varnishes, cleaning products, floor waxes and polishes, adhesives, and metal cleaners.

Based on two studies [52, 57], passively monitored average styrene concentrations ranged from 0.05-0.58  $\mu\text{g}/\text{m}^3$  at home, 0.08-0.48  $\mu\text{g}/\text{m}^3$  in workplaces and schools, 0.28-0.56  $\mu\text{g}/\text{m}^3$  in restaurants and bars, 0.46  $\mu\text{g}/\text{m}^3$  in commercial shopping areas, 0.11  $\mu\text{g}/\text{m}^3$  outdoors along streets, 0.18-0.33  $\mu\text{g}/\text{m}^3$  in public transportation stations, and 0.43-0.45  $\mu\text{g}/\text{m}^3$  in vehicles (private and public). These, and other, results suggest that both indoor and outdoor microenvironments are important determinants of actual styrene exposure [58].

People usually encounter substantially higher VOC concentrations during their normal daily activities compared to the ambient VOC levels recorded at central monitoring sites [58]. A VOC monitoring study in the U.K. examining percentage contribution of microenvironments to personal exposure, reported that home styrene

exposure accounted for 64%, work for 14.1%, and transportation combined accounted for approximately 5.5% of personal exposure [52]. Outdoor microenvironments had negligible contributions.

Styrene exposure due to cigarette smoking is estimated to be 6 ppb annually, with an estimated delivery of 6  $\mu\text{g}$  of styrene per cigarette [59]. Several studies suggest that exposure to styrene is approximately fourfold higher for smokers than for nonsmokers [1, 51, 59-65]. Among smokers, cigarettes are considered the dominant source of styrene exposure. Indoor air styrene concentrations are significantly higher in the homes of smokers than in those homes without smokers [1]. While tobacco smoke is a major source of styrene exposure among smokers, environmental tobacco smoke contributes only about 8% of nonsmokers' exposure [20]. Most styrene exposure derived from environmental tobacco smoke occurs to individuals living with smokers, or those who stay overnight in the presence of smoking. Individuals who spend significant amounts of time (e.g., working) in spaces that formerly allowed smoking, may now experience meaningfully reduced exposure opportunity due to smoking bans [61].

In recent years, indoor styrene concentrations have fallen in many buildings, as a result of reduced or eliminated tobacco smoke, use of low-VOC paints, and other indoor air quality improvements. Analyses of long-term ambient styrene trends indicate that year-to-year regional variation in concentration is not substantial, suggesting that lifetime average estimates are appropriate assessments of exposure [20]. These findings are corroborated by stable measured blood styrene levels in independent U.S. population samples, measured cross-sectionally, over a 20-year period [25, 60, 66].

## **Biological monitoring**

In addition to ambient air monitoring, styrene exposure is measured in blood, exhaled personal air, and as urinary metabolites [25]. In general, these biomarkers correlate well within indoor air styrene levels, but the relationship does not persist for outdoor air styrene concentrations. Observed correlations between environmental and personal measures were higher for biomarkers obtained in occupational settings, which are 25 times higher than average levels in the general population [5, 24].

Styrene is measured in blood in its unmetabolized form (blood styrene), as styrene-7,8-oxide, and by quantifying the formation of DNA and protein adducts [67-70]. Despite the extensive use of adducts as markers of styrene exposure in animal studies, they are less common in human studies. This is, in part, because the capacity of humans to form styrene oxide is much lower than rats or mice, resulting in very low levels of styrene adducts in human blood [71].

For the proposed research, I will use unmetabolized styrene in blood measured using mass spectrometry as a biomarker of personal styrene exposure. The half-life of styrene in blood is approximately 13 hours [5], so blood styrene levels reflect recent exposure. In occupational studies, the correlation between styrene in blood and indoor air ranged from 0.62 to 0.94 [14, 46, 72]. In a study of low occupational styrene levels measured in air, blood, and urine, the best correlations with ambient levels were observed for blood styrene [45]. Between-worker variability is consistently higher than within-worker variability of blood styrene [6, 73].

Recent NHANES data indicate that approximately 40% of the U.S. adult population has measurable levels of styrene in their blood [24, 62]. Samples from

NHANES and other small studies in the U.S. reported similar blood styrene levels that have remained fairly stable over the last 20 years [60, 66, 74]. In RIOPA, blood styrene demonstrated stronger correlation with indoor than outdoor air concentrations, but outdoor sources explained more variability in levels than did indoor sources [61, 75]. The median blood styrene exposure source fractions were 0.58 and <0.01 for outdoor and indoor sources, respectively [75]. In a study of adults in three U.S. urban areas with unique VOC source regimes, personal air samples explained less than 10% of the variance in corresponding styrene blood levels [58]. In a non-occupational setting, pronounced differences in blood styrene levels were seen between smokers and non-smokers (mean, 0.12 vs 0.02 ng/mL, respectively), but no such differences were observed for the presence and absence of environmental tobacco smoke at home among non-smokers [62]. Further, outdoor exposure to environmental tobacco smoke or vehicle exhaust in urban areas has not been associated with increased blood styrene levels [62, 76].

Air styrene exposure levels monitored in environmental studies were highly variable, though determinants of relative exposure remained consistent [58, 59, 62, 75, 77]. Exhaled styrene correlates significantly with indoor air styrene, but not with outdoor styrene levels. The ratio of exhaled to indoor styrene concentrations was close to 1 [58, 75]. The ratio of indoor to outdoor air styrene levels ranged from 3.0 to 5.0, and increased with higher exposure [58, 59, 75]. Higher overnight personal air styrene levels implicate sources or activities within the home as the principal predictors of styrene exposure. In contrast to styrene measured in blood, personal air styrene levels do vary significantly with exposure to environmental tobacco smoke.

Glycol derivatives (mandelic acid (MA), phenylglyoxylic acid (PGA), and hippuric acid (HA)) account for over 95% of the styrene urinary metabolites excreted by humans [1]. The application of these urinary metabolites as styrene biomarkers tends to be more widespread in occupational studies. This may be because they are also used to quantify toluene, ethylbenzene, and other VOC exposure [78-80]. As such, the urinary metabolites MA, PGA, and HA are more appropriate for occupational settings with known co-exposures. Their utility in environmental styrene studies would be potentially limited by exposure misclassification due to co-exposure with other pollutants.

Table 1. Blood styrene levels (ng/mL) in the United States, 1988-2008.

Cohort	Location	Year	N	% Detect	Geo.	Arith.	Med.	P95
NHANES III [60, 66]	United States	1988-1994	657	87.9		0.08	0.04	0.18
LRGVES [81]	Texas	1993	16	50.0		0.07	0.02	0.18
NHEXAS [62]	Midwest states	1995-1997	151	53.0		0.05	0.02	
NHANES	United States	1999-2000	276	94.2	0.04	0.10	0.04	0.20
DREAMS [82]	Minnesota	2000-2002	48	76.2		0.07	0.07	0.11
NHANES	United States	2001-2002	922	54.5	0.04	0.23	0.02	0.20
ATSDR [83]	Louisiana	2002	111	39.0	0.03		0.02	0.06
NHANES	United States	2003-2004	1,213	41.6	0.03	0.04	0.02	0.11
NHANES [15]	United States	2005-2006	1,939	41.5	0.03	0.05	0.02	0.14
NHANES	United States	2007-2008	2,231	37.3	0.03	0.05	0.02	0.14

% *Detect*, Percent of samples above limit of detection; *Geo.*, geometric mean; *Arith.*, arithmetic mean; *Med.*, median; *P95*, 95<sup>th</sup> percentile



## **Determinants and predictors of exposure**

Several different environmental, behavioral, and social factors have been identified as determinants of styrene exposure. It is worth noting that, even in combination, these factors typically explain less than 25% of the variance in measured styrene exposure [52, 59]. This may be due to the fact that styrene is cleared from the body rapidly, and ascertainment of these determinants does not necessarily correspond to the relevant exposure time period. Exposure variation appears to be driven largely by house-level characteristics, rather than seasonal, neighborhood, or measurement variability [61]. Relative importance of different predictors varies depending on the exposure matrix (air, blood, etc.).

Smoking is the single most important individual predictor of human exposure to styrene [1, 20, 51, 59, 60, 64, 84, 85]. The intake of styrene due to smoking 20 cigarettes outweighs the total daily intake from food and air [23]. Exposure to environmental tobacco smoke is also associated with higher styrene exposure, particularly for non-smokers living with a smoker [59, 85]. Working in an environment with environmental tobacco smoke less consistently predicts styrene exposure. Among NHANES III participants, consuming more than one alcoholic beverage a day, on average, was also associated with increased blood styrene exposure [66].

Housing materials and characteristics are strongly predictive of VOCs, and styrene in particular. The size of the house (number of rooms) and number of windows are inversely associated with styrene exposure [75]. The house air exchange rate is negatively associated with styrene levels [86]. Likewise, opening windows and doors is predictive of lower styrene exposure. Conversely, air fresheners, excessive dust,

majority of floors carpeted, fireplaces, gas heating source, and attached garages are all associated with increased styrene exposure [51, 52, 59, 75]. Parking and storing motor vehicles and gas-powered equipment in the garage are also associated with increased styrene exposure [75]. Time spent at home is inversely associated with styrene exposure, suggesting that activities and sources outside the home could be driving higher exposures in non-smokers [75].

Ambient relative humidity and wind speed are inversely associated with indoor styrene levels [87]. That is, dry, still conditions predict higher indoor air styrene. Wind speed dilutes outdoor concentrations from local sources and increases household air exchange rate, which effectively reduce indoor and outdoor air styrene exposure. Outdoor relative humidity may be a surrogate for precipitation, possibly representing the effects of low pressure systems with good dispersion or effective cleansing [75].

Blood styrene levels are fairly consistent within season, though monitoring suggests significantly lower styrene levels in the spring compared to all other seasons [56]. This is consistent with meteorological conditions (wind speed and humidity) that favor lower ambient concentrations. Warmer months are characterized by increased seasonal emissions, more active atmospheric oxidation chemistry, and elevated outdoor styrene concentrations [87]. Relatively higher winter and autumn levels are likely attributable to increased rates of combustion for heating, lower photochemical degradation, and low mixing heights in cold seasons. Seasonal variability in personal styrene exposure may be difficult to predict because of the interaction between meteorological conditions and behavioral response. In the Gulf region, where hot summer temperatures potentially cause elevated outdoor concentrations, personal

exposures are influenced by the amount of time spent inside, and the use of air conditioning or windows.

Regional variability of styrene levels between urban and rural areas suggests that differing outdoor source regimes and traffic emissions impact individual exposure [66, 75]. Other factors that might influence this relationship include weather and climate conditions, proximity to roadways, regional patterns in housing construction, and time spent in motor vehicles. Source apportionment factor analyses of the RIOPA data show that styrene, toluene, ethylbenzene, and xylenes form a common exposure mixture that accounts for 20% of total VOC exposure, and is attributed to motor vehicle exhaust [87].

In population-based studies, reported occupational exposure to paint, cleaning products, and degreasers were associated with elevated styrene exposure [51, 59, 75]. Working in plastics, chemical, paint, or dye plants, as well as metal working, increased odds of high styrene exposure [51, 59]. These exposures encountered in presumably lower frequency or duration, through being in a paint store, or building scale models, painting, and doing metalwork as hobbies similarly predicted elevated styrene exposure. Spending time near vehicles or engines, in any capacity, was associated with higher breath styrene levels among non-smokers [75].

Demographic predictors of increased styrene exposure include race, ethnicity, and male gender. Non-Hispanic white people were significantly more likely than Mexican Americans to experience elevated styrene exposure, though no differences were seen for comparisons of white and black people in NHANES III [66]. A previous study demonstrated higher styrene levels among non-white participants than white participants [51]. I hypothesize that these characteristics indirectly determine styrene

exposure through the previously discussed occupational exposure opportunities, housing characteristics, behavioral determinants, and proximity to outdoor sources.

Though ingestion of styrene is a possible exposure route, examinations of diet, weight, and body mass index as determinants of styrene exposure remain absent from the literature. Because increased styrene migration is associated with higher fat content [30], it is possible that a high-fat diet or elevated BMI would be predictive of styrene exposure. However, the trace amounts of styrene in food appear to be swamped by inhalation exposure, so the proportion of exposure due to ingested styrene is likely difficult to quantify [24].

Although a candidate set of predictors for styrene exposure exists (smoking, environmental tobacco smoke, housing characteristics, and occupational exposures), the estimated relative contributions of these predictors, and others, are inconsistent. Further, the impact of outdoor sources and styrene concentrations on personal exposure levels remains unclear. Despite higher indoor concentrations, outdoor sources explained more variability in personal exposure measurements (median outdoor exposure source fraction, 0.58) than indoor sources (median indoor exposure source fraction, <0.01) in the Relationship of Indoor Outdoor and Personal Air (RIOPA) study [61, 75, 77]. Additionally, the presence or absence of a styrene source was a stronger determinant of indoor air concentration than the air exchange rate in one study [59]. The lack of consensus may be partly due to incomplete source ascertainment in many studies, which typically prioritize either indoor or outdoor styrene sources, but don't comprehensively assess both. The proposed study includes detailed indoor exposure information, as well as two different assessments of outdoor styrene levels and sources.

## **B. Potential health effects of styrene exposure**

### **Toxicity**

In humans, approximately 70% of inhaled styrene is absorbed [6]. Styrene is distributed throughout the body, with the highest concentration generally found in adipose tissue [1]. Metabolism of styrene occurs primarily in the liver via the styrene 7,8-oxide pathway. It is then excreted in the urine as mandelic and phenylglyoxylic acids [88]. After inhalation exposure, styrene can be metabolized in the lungs and nose, resulting in toxicity and, less commonly, carcinogenicity [89]. Styrene metabolism is concentration-dependent, with metabolic saturation occurring at 140–280 ppm in humans [90].

The health effects of styrene have been studied primarily in experimental animal studies and occupational human studies. Occupational exposure to styrene has been associated, albeit inconsistently, with a variety of acute health effects. Irritation of the eyes, throat, and respiratory tract, and contact dermatitis [91] have been reported, but not replicated [1]. Respiratory conditions, including chronic bronchitis [92] and asthma [93, 94], and hematological changes [95] have been demonstrated in highly exposed styrene workers. Occupational styrene exposure is inconsistently associated with altered liver function and elevated serum bile concentrations [6, 96, 97]. In animal studies, styrene exposure causes liver and lung toxicity in mice and nasal toxicity in rats and mice [71].

Based on limited evidence in humans and experimental animals for the carcinogenicity of styrene, the International Agency for Research on Cancer (IARC)

classifies it as possibly carcinogenic to humans (Group 2B) [6]. Data from both laboratory and human occupational studies demonstrate that styrene exposure leads to formation of DNA adducts, DNA damage, and cytogenic effects [68, 70, 71, 98]. In styrene-exposed workers, levels of DNA adducts are up to five times higher than those in non-exposed controls [98].

Though styrene's carcinogenic and genotoxic properties have been well characterized [6], the Agency for Toxic Substances and Disease Registry (ATSDR) has identified the central nervous system as the primary target for styrene toxicity, with less marked effects in the peripheral nervous system [2, 99].

### **Neurotoxicity**

Styrene monomer functions like many other VOCs, depressing the central nervous system and exhibiting anesthesia-like properties [6, 100]. Solvent-induced neurotoxicity, including that caused by styrene, produces symptoms of acute intoxication, commonly described as a feeling of drunkenness. Short-term acute intoxication is reversible, ceasing when styrene is cleared from the body. More concerning for overall health are the potential chronic, subtle but demonstrative, and irreversible effects that persist after styrene is cleared from the body [101]. It is not clear whether irreversible neurotoxic effects of styrene occur, and at what exposure levels.

Occupational studies demonstrate styrene-induced neurotoxicity, evident as central and peripheral nervous system effects, from both acute and chronic inhaled exposure among highly-exposed workers. Symptoms of neurotoxicity, including feeling "drunk" and tiredness [41], impaired vision [99, 102], vestibular dysfunction [12],

headaches [103], delayed reaction time [104, 105], impaired attention and memory [9], hearing deficits [106], diminished nerve conduction velocity [9, 107-110], and abnormal EEG results [110, 111]. Dopaminergic [41, 112-114], functional [115, 116], and psychiatric anomalies [103, 117] have also been associated with high occupational exposure (approximately 100 ppm) to styrene. Similar effects have been observed at lower occupational exposure levels, ranging from 10-30 ppm, in most [10, 99, 103-105, 114, 117-119], though not all [13, 120], studies. Increased mortality from diseases of the central nervous system, especially epilepsy, was associated with styrene exposure in a cohort study of reinforced-plastics industry workers [121].

Recent environmental studies evaluating effects of simultaneous exposure to multiple hazardous air pollutants have documented associations between environmental styrene and neurologic outcomes. When evaluating modeled ambient exposure estimates for a variety of HAPs, styrene was associated with increased risk of autism spectrum disorder [122, 123] and amyotrophic lateral sclerosis [124]. A cross-sectional analysis of blood VOCs and neurobehavioral testing in NHANES III found a general lack of significant adverse effects, with the exception that a mixture of BTEX and styrene was modestly associated with slower reaction time [125]. These results suggest that styrene may impact neurologic function at environmental levels relevant to the general population, implicating low-level, chronic styrene exposure as a possible public health problem. The human health effects due to chronic styrene exposure at typical environmental levels among the general population remain largely unknown – due in part to uncertainty regarding the magnitude of these levels and their underlying sources across populations [20]. Examining neurologic symptoms can reveal subclinical

impairments in neurologic function that appear earlier in the progression of disease. While less severe, these symptoms may be more sensitive to lower exposure levels, more prevalent in the general population, and possibly persist after exposure recedes.

## **Mechanism**

Studies in humans and experimental in vitro and in vivo animal models have attempted to determine the mode of action for styrene neurotoxicity, with a dopaminergic mechanism gaining traction [71], but explanations remain speculative. Several studies suggest that styrene exposure alters dopamine metabolism, marked by decreased dopamine levels and increased dopamine receptors in rodents and humans [126-128]. The styrene metabolites phenylglyoxylic acid and mandelic acid were shown to deplete dopamine [129]. This mechanism is corroborated in blood samples of styrene-exposed plastics workers for whom prolactin levels are elevated, as prolactin release from the anterior pituitary gland is chronically inhibited by dopamine [130]. The change in prolactin may signal diminished inhibition of prolactin release by dopamine, whether through lower dopamine levels or weakened inhibitory action [127]. A dose-dependent decrease in monoamine oxidase B was also observed in rats and workers exposed to styrene [41, 112].

Consistent with disturbance of the dopaminergic functions of the brain, styrene exposure potentiates a dose-dependent decrease in brain dopamine and an increase in homovanillic acid in male rats [128]. A decrease in dopamine and its metabolites in the corpus striatum, hypothalamus, and the lateral olfactory tract regions of the brain was observed in rats that were highly exposed to styrene [131]. Loss of motor function,



lasting for approximately a month, accompanied these changes in dopamine. This physiological manifestation supports the hypothesis that dopamine mediates styrene exposure to impact neurologic function. Styrene also caused cell loss and dopamine depletion in retinas isolated from female rats [132], which supports the established association between occupational styrene exposure and impaired color vision [133].

### **C. Summary of Background and Significance**

The blood styrene levels observed in the GuLF STUDY are much lower than occupational levels, but two to three times higher than those observed in the general U.S. population. This difference persists regardless of cigarette smoking, suggesting meaningfully increased exposure opportunity in the region. Identifying sources of uniquely elevated styrene exposure has implications for exposure mitigation policy.

Given that styrene use is widespread, it is a critical public health priority to evaluate whether adverse health effects result from chronic exposure at environmental levels. Quantifying the association between environmental styrene exposure and highly sensitive, but non-specific, neurologic symptoms may lend insight into early manifestations of environmentally induced neurotoxicity. This research provides insight into the underlying sources responsible for styrene exposure in this population, as well as being the first to examine neurologic effects of styrene exposure of this magnitude.

## CHAPTER III: METHODS

### A. Study Design and Population

#### GuLF STUDY

This research builds upon the GuLF STUDY, a prospective cohort study of community members who participated in the cleanup of the Deepwater Horizon oil spill in 2010 and comparison subjects who did not, for which extensive data and banked biospecimens have already been collected. Of the 32,608 people who enrolled in the GuLF STUDY between March 2011 and June 2013, 26,828 (90% of those contacted and eligible) resided in one of the five Gulf States (Alabama, Florida, Louisiana, Mississippi, and Texas). For reasons of exposure opportunity and data collection logistics, the proposed dissertation research is restricted to these 26,828 participants living in the Gulf States.

All English- and Spanish-speaking cleanup workers and a random sample of non-cleanup workers residing in one of the Gulf States were additionally invited to participate in a home visit, which included extensive interviewing on lifestyle, occupational, and health factors, collection of anthropometric measures, and blood collection. Over 11,000 participants (66% of those invited) completed a home visit. A stratified random sample of 1,055 home visit participants, oversampled for women and non-smokers, was recruited for a Chemical Biomonitoring Study (CBS) (94%

participation) at the time of their home visit two to three years after the oil spill. These CBS participants provided an additional 10 mL tube of whole blood for measurement of VOCs, including styrene. The whole blood specimens were shipped overnight at 4°C to the Centers for Disease Control and Prevention (CDC) for analysis. GuLF STUDY personnel recorded the latitude and longitude of the home visit location using a handheld Global Positioning System (GPS) device. Home visits among CBS took place in 2012 and 2013, a median of 105 days after enrollment, with 26% of exposure biomonitoring visits completed within 1 month of enrollment and 47% completed within 3 months of enrollment. Ultimately, after removal of samples due to insufficient quantity (N=13) and assay failure (N=47), 994 participants provided blood samples sufficient for quantification of styrene levels.

Approximately 3,400 home visit participants living within 60 miles of New Orleans, LA or Mobile, AL completed a clinical examination between August 2014 and July 2016. Examinations took place in two clinical settings (one in New Orleans, LA and one in Mobile, AL) and included anthropometric measurements, biological sample collection, standardized computer-assisted neurocognitive testing, peripheral neurologic function evaluations, pulmonary function testing, and mental health questionnaires.

Analyses rely on data collected at enrollment from 26,828 participants in the Gulf States, during the CBS home visit from 994 participants, and at the follow-up clinical exam from approximately 3,400 participants. Both the CBS and the clinical exam sub-populations are nested within the Gulf States portion of the parent GuLF STUDY population.

## **Human Subjects**

The GuLF STUDY received approval from the Institutional Review Board of the National Institute of Environmental Health Sciences. This dissertation utilizes previously collected, de-identified data and no study participants were contacted for additional information. The project was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill.

## **Eligibility and sampling for Specific Aims 1 and 2**

Analyses for Specific Aims 1 and 2 employ data collected at enrollment and the CBS home visit, as well as spatially referenced exposure assignment. As such, these analyses are restricted to those 994 GuLF STUDY participants who provided blood specimen for styrene measurements during participation in CBS. Because exposure assessment for these analyses is location-based, any members without a plausible geocoded location are excluded. Eligible participants in these analyses meet the following criteria:

- Residence in one of the five Gulf States
- Completion of home visit and provision of blood styrene specimen
- English- or Spanish-speaking
- Plausible geocoded home location
- Quantifiable blood styrene and 2,5-dimethylfuran samples
- Complete covariate information

Of the 994 participants with measurable blood styrene levels, 935 also have measured blood 2,5-dimethylfuran (2,5-DMF) levels. 2,5-DMF is a VOC and validated

blood biomarker for smoking [64]. Those 935 CBS participants with blood styrene and 2,5-DMF information comprise the sample for comparisons to NHANES.

For regression analyses predicting blood styrene levels, we further restrict to participants who completed the recent exposure questionnaire (n=810) and have complete demographic and covariate information, yielding a final analytic sample of 667 participants.

### **Eligibility and sampling for Aim 3**

Aim 3 analyses examine associations between environmental styrene exposure and neurologic effects. Ambient styrene exposure is characterized using NATA concentrations, and blood styrene exposure is measured from samples provided in CBS. Neurologic outcomes include self-reported symptoms collected at enrollment and peripheral neurologic function testing conducted at follow-up. We exclude participants reporting physician diagnosis of diabetes from all neurologic analyses because peripheral neuropathy is a known complication of diabetes.

Eligible participants for the analyses of ambient styrene exposure and neurologic outcomes meet the following criteria:

- Residence in one of the five Gulf States
- English- or Spanish-speaking
- Plausible census-based home location
- Complete outcome ascertainment
- Complete covariate information
- No self-reported physician diagnosis of diabetes

Of the 26,828 English- or Spanish-speaking GuLF STUDY participants residing in the Gulf states, 25,844 reported addresses which returned plausible census locations for ambient exposure assignment. We exclude any participants missing any symptom (n=1,245), demographic (n=573), or covariate (n=201) information, as well as anyone with known or missing diabetes diagnosis. Ultimately, this analysis of ambient styrene in relation to neurologic symptoms includes 21,962 non-diabetics with complete exposure, outcome, and covariate information.

The association between ambient styrene and peripheral neurologic function includes 2,956 participants out of 3,403 who completed the follow-up clinical exam. Eligibility for participation in the follow-up clinical exam was restricted to GuLF STUDY participants living within 60 miles of New Orleans, LA or Mobile, AL. We made exclusions for implausible census location (n=74), missing all neurologic function testing information (n=8), missing covariate information (n=58), and possible diabetes diagnosis (n=305).

Eligibility requirements for analysis of associations between measured blood styrene levels and neurologic outcomes (both symptoms and test performance) are as follows:

- Participation in CBS and provision of blood styrene specimen
- English- or Spanish-speaking
- Plausible census-based home location
- Successful lab measurement of blood styrene
- Complete covariate information

Out of 994 CBS participants providing adequate blood samples for styrene measurement, 974 had complete neurologic symptom ascertainment. We excluded 13 participants with missing covariate information and 87 known or possible diabetics, arriving at a final analytic sample of 874 participants.

The intersection of CBS and the follow-up clinical exam include 343 eligible participants with blood styrene measurements and peripheral neurologic function testing, all of whom had complete demographic and covariate information. We exclude 33 known diabetics, yielding a final sample size of 310 participants for the analysis of blood styrene and peripheral neurologic function.

## **B. Data Collection and Measurement of Variables**

### **Exposure assessment**

Styrene exposure was ascertained 4 ways:

Blood styrene levels

NATA ambient concentrations

NEI point source proximity

Self-reported behavior and lifestyle indicators

For location-based exposure assessment (NATA ambient concentrations and NEI point source proximity), each participant's home residence was geocoded and associated with the corresponding 2010 U.S. census tract within which it is located. Participant residence was ascertained at enrollment via self-reported address and at the

home visit via hand-held GPS device. Reported addresses were geocoded by GuLF STUDY personnel using ArcGIS and the Google maps application program interface.

Approximately 85% of home visits were successfully geocoded using the GPS device, and the remaining 15% were preliminarily geocoded to the corresponding census tract centroid. We preferentially use GPS-generated geocodes in analyses where available. Participants with implausible or missing GPS-generated geocodes were assigned address-based geocodes. Participants for whom no geocode location (GPS or address-based) was successfully determined were assigned to the nearest census tract centroid.

### **Blood styrene levels**

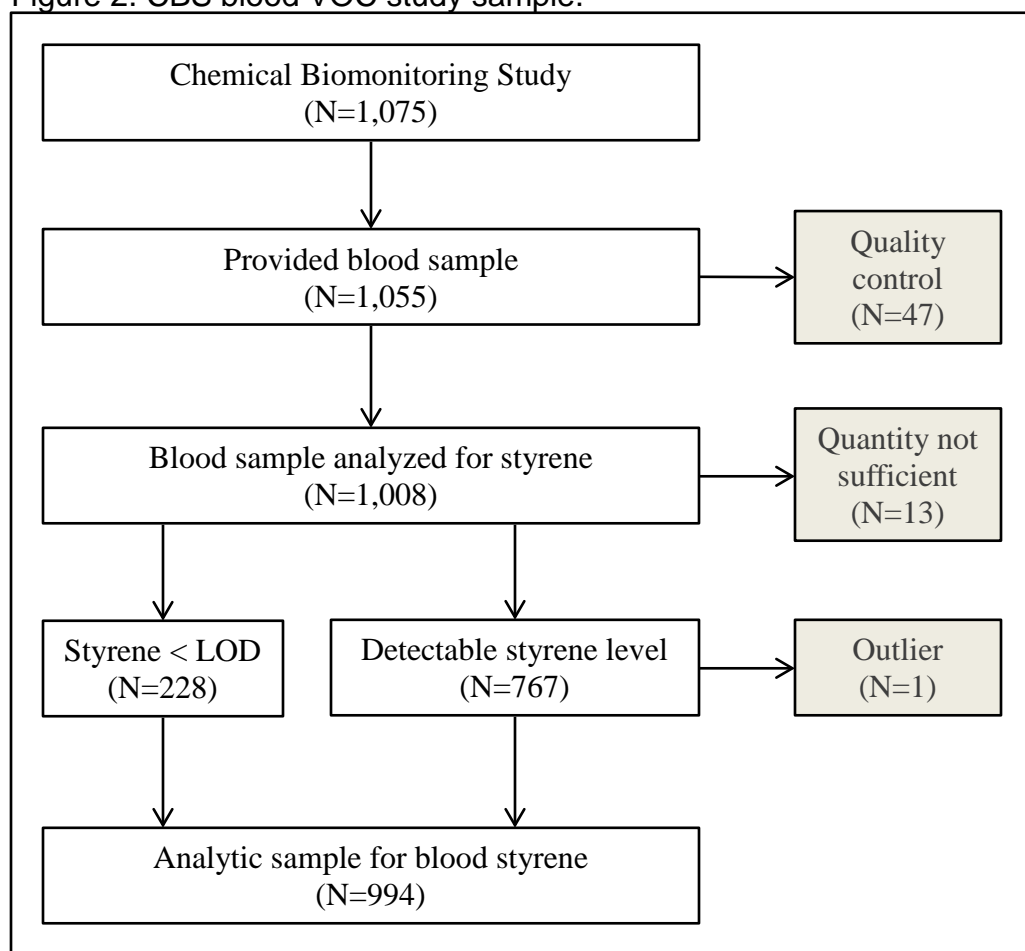
Participants provided a single 10 mL tube of whole blood at the home visit for measurement of VOCs. Measured pollutants included styrene, benzene, toluene, ethyl benzene, and xylenes (BTEX) and others. The whole blood specimens were shipped overnight at 4°C to the Centers for Disease Control and Prevention (CDC) for analysis.

Blood levels of styrene and other chemicals were measured at the CDC laboratory using the standard methods used in NHANES. Measurement was by equilibrium headspace solid-phase microextraction/gas chromatography/mass spectrometry of hermetically collected blood specimens [134, 135]. Of the 1,055 recruited CBS participants who provided blood specimen for any blood VOC measurements, 13 were eliminated because the quantity of sample was insufficient to measure styrene, 47 were removed due to quality control failure, and one outlying value (15.3 ng/mL) was eliminated. The remaining 994 samples, including those below the



limit of detection, make up the sample size for analyses examining blood styrene levels. Approximately 80% of samples had measurable styrene levels above the limit of detection. The laboratory provided all measured values, including those below the limit of detection (0.03 ng/mL).

Figure 2. CBS blood VOC study sample.



### National Air Toxics Assessment 2011

The EPA's 2011 National-scale Air Toxics Assessment (NATA) evaluates 180 air toxics across the United States using emissions inventories, dispersion modeling, photochemical modeling, exposure modeling, and toxicity analyses. In this most recent

NATA release, styrene is modeled using the dispersion component of the Human Exposure Model, version 3 (HEM-3). HEM-3 is a computer model designed to conduct inhalation risk assessments for sources emitting air toxics to ambient air. HEM-3 contains the American Meteorological Society (AMS) and EPA Regulatory Model (AERMOD) dispersion model for air-transport simulations and U.S. Census data for identifying population receptors. AERMOD contains emissions and meteorological data, which is combined with HEM-3 census data to generate annual average ambient air toxic concentrations for each U.S. census tract.

Emissions inputs to AERMOD come from the 2011 National Emissions Inventory. AERMOD predicts annual census tract concentrations for each major source type (point, nonpoint, on-road, non-road), which are then summed to a total concentration value for all source types combined.

NATA has been widely evaluated against observed air monitoring data in many sites across the country, both externally [136-143] and by the EPA [144]. AERMOD performance varies by pollutant, and styrene demonstrates strong agreement with monitored data in the Gulf region for 2011, based on a correlation of 0.73 and a model to monitor concentration ratio of 1.1. Assuming observed monitor values represent true ground-level exposure, AERMOD appears to characterize ambient styrene sufficiently well for use as an indicator of human exposure.

Air toxics monitoring lacks the spatial and temporal coverage to estimate or interpolate exposures with any reasonable degree of certainty, particularly when compared to the residential locations of GuLF STUDY participants (Appendix 1). The average distance to the nearest styrene monitor among CBS participants is 114 miles,

and only 31 CBS participants within 10 miles of a monitor. The contribution of monitored styrene via kriging or otherwise combining with NATA would be negligible [145].

We use NATA styrene estimates as indicators of typical, long-term environmental exposure. Although the assumptions inherent to an annual average estimate of air pollution potentially limit interpretation for acute exposure scenarios, NATA data are a valid estimation of usual exposure levels experienced in the Gulf. Despite the loss of spatial resolution within census tracts, dispersion modeling is considered superior to distance-based exposure metrics because it integrates meteorology, comprehensive ascertainment of all source types, and weighting by emissions quantities [146, 147].

### **Spatially estimated styrene exposure: National Emissions Inventory 2011**

Point sources of styrene emissions were identified using the 2011 National Emissions Inventory Version 2 (NEI), the EPA's latest comprehensive database of annual criteria (CAP), precursor, and hazardous air pollutant (HAP) emissions. The Air Emissions Reporting Rule (AERR) requires jurisdictions to report all sources of CAP and precursor emissions, including total VOCs, and sets forth guidelines for voluntary reporting of HAPs. Sources are collected from state, local and tribal air agencies, and augmented with information from the Toxics Release Inventory (TRI), the Acid Rain Program, and EPA's regulatory air toxics data. The NEI is compiled and released every three years.

NEI sources are broadly categorized as stationary or mobile emitters. Stationary emissions sources, which are further designated as point or nonpoint, include large industrial sources (power plants and refineries), smaller industrial and commercial

sources (dry cleaners and commercial cooking), and residential sources (residential wood combustion and consumer products usage). The point and nonpoint designations reflect the way each source is modeled – point sources are associated with a latitude-longitude geographic coordinate location, whereas all other sources are aggregated to county totals. The emissions potential of each stationary facility determines its designation as a point or nonpoint source, according to emissions thresholds set in the AERR. For the majority of the five-state GuLF STUDY region, the point source emissions threshold is 100 potential tons of ozone per year. The remaining study area includes two ozone non-attainment areas, where point source designation is attributed to facilities with the potential to emit at least 50 tons of ozone annually.

Nonpoint (stationary facilities emitting below the AERR threshold), on-road (cars and trucks driven on roads), non-road (locomotives, aircraft, marine, construction vehicles, off-road vehicles, and lawn and garden equipment), and event (wildfires, prescribed burning) sources are reported as aggregated county totals for each source.

In Specific Aim 2, we focus exclusively on point sources. Styrene emissions are estimated directly if a facility voluntarily reported HAPs, or indirectly for those facilities and jurisdictions that do not report HAP emissions. Indirectly reported styrene emissions are calculated by multiplying the appropriate surrogate CAP emissions volume by an emissions factor associated with the industrial process of interest. For all styrene emissions, the CAP surrogate is total VOCs. The emissions factors vary depending on the Source Classification Code (SCC) of each process. We abstracted styrene point source locations by identifying all records of reported styrene emissions from the 2011 NEI point source database.

## **Self-reported styrene exposure**

GuLF STUDY participants provided self-reported information that is potentially predictive of styrene exposure a minimum of one time (baseline telephone questionnaire at enrollment), and a maximum of three times (in-person home visit questionnaire, in-person exposure biomonitoring questionnaire at home visit).

Extensive, thorough, detailed information about exposure to cigarette smoke (both active and passive) was obtained at enrollment and the home visit.

All GuLF STUDY participants in the five-state Gulf region (N=26,828) completed the enrollment Computer Assisted Telephone Interview (CATI). Information obtained here is applied to all three Specific Aims.

All CBS participants also completed the home visit questionnaire at the time of their blood draw. Information obtained here is applied in Specific Aims 1 and 2. This instrument is used primarily to characterize residential and recreational styrene exposure opportunities. A complete residential history was compiled including all places the participant lived for at least 3 months during their life. Residential characteristics included:

- Live within ½ mile of a major highway, boatyard, docks, oil refinery, petroleum storage or transfer facility, gas stations, factory, power plant, hazardous waste site or Superfund site, landfill
- Years lived at residence
- Usual water supply at residence
- Residential proximity to center or margin of town (urban/rural)
- Residence classified as farm

Non-occupational exposure opportunities were assessed by asking, *Do you have any of the following hobbies?*

- Woodworking or cabinetry, Boat repair, Car, motorcycle, or other vehicle repair, Gardening, Fishing, Pottery, Painting as art work, Sculpture, Home repairs or handyman work, Raising farm animals

All CBS participants completed the corresponding recent exposure questionnaire at the time of the blood draw. This instrument was developed to obtain detailed information about relevant predictors of VOC and metal exposure, though styrene was not specifically prioritized. Information obtained here is used in Specific Aims 1 and 2. This instrument is used primarily to characterize residential and non-occupational styrene exposure opportunities in the immediate vicinity and time of the blood collection.

The following information pertains to housing and microenvironmental exposures:

- Year built, housing type, number of rooms, exterior wall construction, attached garage, garage vehicle and equipment inventory
- Home renovations in the past 6 months
- New furniture in the past month
- Anyone painted in or around the home in the past 6 months

Activities performed by anyone in or around the home:

- In the past 24 hours: cook, clean, burn candles or incense, open windows, open exterior doors, burn fuel
- In the past week: painting, using chemical paint strippers, adhesives, or home maintenance products (caulk, grout), doing metalwork

- *Did you smell smoke or any unusual chemical smells in or around your home within the last 24 hours?*

Products and materials used in or around the home in the past 24 hours:

- Cleaning products, aerosol spray products, air fresheners, petroleum-based solvents, paints or glues, household pesticides or lawn chemicals, air purifier, air filter, heater/heating devices, air conditioner, window fan
- Use and repair of lawn mowers, small engines, equipment, and machinery

Vehicle use in the past 24 hours):

- Time driving, riding in, and refueling gas-powered and diesel vehicles

Time spent away from home in the past 24 hours:

- Hours away from home, hours outside, hours exercising outside

The following information about current occupation at the time of the home visit was obtained:

- *Do you usually work a total of 35 hours or more per week?*
- *Did you work yesterday or today before this visit?*
- Hours worked, commute type, and commute time in the past 24 hours
- Materials worked with or near in the past 24 hours: insulation, brake shoes, paints, varnishes, stains, or strippers, other chemical used to clean floors, walls and other surfaces, diesel engine exhaust, gasoline engine exhaust, pesticides

## **Outcome assessment**

Neurologic outcomes were ascertained two ways:

Self-reported neurologic symptoms

Peripheral neurologic function testing

## **Self-reported neurologic symptoms**

Detailed health outcome ascertainment, including questions about neurologic symptoms, was completed at enrollment via CATI during the baseline interview.

Symptoms of neurologic function were reported with respect to the previous 30 days.

Participants were asked to report how often they experienced dizziness,

lightheadedness, vision impairment, numbness, tingling, loss of balance or stumbling,

headaches, and fatigue during this time period. Three additional outcomes, insomnia,

vomiting, and seizures, were added to the questionnaire after data collection was

underway. These outcomes are combined with other symptoms analytically only in

appropriate population subsets because their enumeration in the study population is

incomplete (i.e., those people who completed the enrollment interview prior to the

inclusion of seizures and insomnia did not have the opportunity to report them).

Questionnaire items were developed using previous literature describing symptoms of petroleum/VOC-based neurotoxicity. All symptoms in question have been

investigated with occupational styrene exposure and demonstrated significant

associations, with varying degrees of consistency [1, 6, 24]. Symptoms of neurologic

function were ascertained as follows:



*In the past 30 days, how often:*

- *have you had a severe headache or migraine?*
- *have you felt dizzy or lightheaded?*
- *have you been nauseated?*
- *have you experienced vomiting?*
- *have you had blurred or distorted vision?*
- *did you have tingling or a “pins and needles” feeling in your hands, arms, feet, or legs?*
- *you have numbness (parts of your body “go to sleep” for no apparent reason) in your hands, arms, feet, or legs?*
- *did you stumble while walking?*
- *had excessive fatigue or extreme tiredness?*
- *have you experienced seizures?*
- *did you have insomnia?*

Frequency of symptom occurrence was reported as: *all of the time, most of the time, sometimes, rarely, never*. Symptoms are analyzed as a binary indicator of the presence (all or most of the time) or absence (sometimes, rarely, or never) of occurrence. A lower threshold is applied to the episodic symptoms because the relative frequency of their occurrence is expected to be lower, as compared with potentially ongoing symptoms. Accordingly, the presence of vomiting includes responses of sometimes, most, or all of the time, and the presence of seizures includes reporting a seizure at any point during the 30 days (rarely, sometimes, most of the time, all of the time). Adjusting thresholds in this manner is meant to facilitate interpretation of results.

## **Peripheral neurologic function testing**

Follow-up clinical examinations included anthropometric measurements, biological sample collection, peripheral neurologic function testing, neurobehavioral evaluations, pulmonary function testing, and mental health questionnaires. The neurologic function testing battery, which has been implemented in a previous study of chronic, low-level neurotoxicants [148], included the following seven tests: visual acuity, visual contrast sensitivity, handgrip strength, vibrotactile threshold testing, postural stability, single leg stance, and long-distance corridor walk. This study will focus on a subset of the neurologic function tests, selected to correspond with effects of solvent-induced neurotoxicity in the occupational literature [149, 150].

*Visual contrast sensitivity* was evaluated with the Functional Assessment of Contrast Sensitivity test using a standard testing instrument, the Optec 1000 (Optec, Inc. U.S.A). Circular stimuli consisting of alternating light and dark bars were presented. Nine stimuli of decreasing contrast were presented at each of 5 spatial frequencies, i.e., 1.5, 3, 6, 12, and 18 cycles per degree. The index of the weakest contrast correctly identified (i.e., threshold) was recorded for each spatial frequency.

*Postural stability* was evaluated using the Advanced Mechanical Technology, Inc. (AMTI) force platform by measuring the forces applied to the platform through the participant's feet. The device uses strain gauges in the metal platform and a computer interface to record the forces applied to it. The signals from these strain gauges are amplified, digitized, and stored in the computer. From these forces, a times series of locations of the participant's center of pressure can be collected. The path of these center of pressure locations is plotted on the computer screen and the length and

velocity of the sway path over a standard time period (e.g., 60 seconds) is recorded. Summary measures such as the average deviation in the lateral and anterior-posterior directions will also be calculated. The test was repeated two times with the participant's eyes open and two times with eyes closed.

*Single leg stance* was evaluated by asking the participant to stand on one leg and maintain balance for 30 seconds. If the participant was unable to maintain their balance for the entire 30 seconds, the procedure was repeated up to two additional times.

*Vibrotactile threshold* was evaluated using a portable Vibratron II electromechanical vibrometer consisting of a controller unit and two identical transducer units that cause plastic posts protruding from their housings to vibrate at a frequency of 120 Hz. The amplitude of the vibration is controlled manually by the examiner and displayed digitally on the face of the controller. We obtained five threshold values (three descending and two ascending values) for each great toe, requiring a total of about 10 minutes. The final vibration threshold for each toe is the median value obtained from values 2-5 (value 1 is discarded). Examiners entered data manually into the data system.

*Handgrip strength* was assessed with a self-contained mechanical/hydraulic device that records on a dial the maximum force exerted by the participant's "power" or whole-hand grip. It is equipped with a "tell-tale" that retains the maximum excursion of the force indicator needle. We performed one set of three grip strength measures of the dominant hand followed by one set of three grip strength measures of the non-dominant

hand. The bilateral mean of the six measures is the summary metric for the handgrip measure.

### **Covariate assessment**

Covariates were obtained from the enrollment, home visit, and CBS questionnaires. For the predictive modeling in Specific Aims 1 and 2, covariates were selected on the basis of their theoretical association with styrene exposure. For the exposure-outcome modeling in Specific Aim 3, covariates were selected according to their theorized relationships with both the exposure and the outcome. Aim 3 analyses rely on covariates ascertained exclusively from the enrollment questionnaire.

Cigarette smoking is a primary source of population styrene exposure, with reported VOC levels in the blood of smokers mimicking levels found in mainstream cigarette smoke [64]. As such, determining smoking status and factors affecting dose of cigarette smoke received is essential in analyses of styrene as both an exposure (Specific Aim 3) and outcome (Specific Aims 1 and 2). Detailed reported smoking information was collected at each phase of study. Additionally, 2,5-DMF, a biomarker of smoking was measured in blood among all CBS participants. Because mainstream and sidestream cigarette smoke are strong predictors of styrene exposure, we assessed cigarette smoke multiple ways.

Alcohol consumption is associated with personal styrene exposure [66], and acute alcohol intoxication presents as many of the self-reported outcome measures in this study [151]. As such, alcohol consumption is a predictor of both the primary exposure and outcome, and will be treated as a potential confounder. Drinking habits

were ascertained with respect to lifetime habits, the past 12 months, and the past 30 days. Alcohol consumption is considered in all three Specific Aims.

Demographic and social characteristics, including age, sex, marital status, race, and ethnicity were ascertained at enrollment using standard questions. Body mass index was calculated based on reported height and weight at enrollment, and measured height and weight at the home visit. These variables are applied in all three Specific Aims.

Socioeconomic status is potentially associated with many predictors of styrene exposure, as well as health status and likelihood of symptom reporting. Indicators of socioeconomic status were collected at enrollment and the home visit. Highest grade level completed, household income, and current work status were all collected at enrollment.

## **C. Data Analysis Methods**

Specific Aim 1: predict blood styrene levels using GuLF STUDY and NATA data

Specific Aim 2: predict blood styrene levels using NEI data

Specific Aim 3: examine associations between styrene exposure and neurologic outcomes

### **Specific Aim 1**

Because these specimens were collected two to three years after the oil spill and styrene's biological half-life in blood is 13 hours, these measurements reflect contemporary, ongoing exposures. Indeed, we observe virtually no correlation between

individual-level blood styrene levels and working on oil spill cleanup (Table 2). Any variability in styrene exposure according to oil spill cleanup experience is likely an artifact of other lifestyle factors associated with individual styrene exposure. Styrene is derived from, but not an important constituent of, petroleum [1, 78]; therefore, we do not estimate or account for cleanup-related styrene exposure in Specific Aim 1.

Table 2. Bivariate distribution of blood styrene levels stratified by participation in DWH oil spill cleanup work.

	Workers	Non-workers
N	844	150
Mean (SD)	0.31 (2.1)	0.31 (2.1)
Geometric mean (SD)	0.10 (4.0)	0.08 (4.2)
Minimum	0.02	0.02
25th percentile	0.03	0.02
Median	0.07	0.05
75th percentile	0.19	0.16
95th percentile	1.62	2.00
T statistic (p-value)	0.09 (p=0.90)	
R <sup>2</sup>	0.00001	

### **Characterize the distribution of blood styrene with respect to NHANES**

To contextualize the observed blood styrene concentrations in CBS, we compare them to the levels observed in NHANES, examining differences in central tendency and interquartile range of blood styrene levels between the two study cohorts. The presence of outliers, skew, and other deviations from normal distribution is noted.

These comparisons are evaluated jointly with cigarette smoking status.

In addition to comparing the continuous distribution of exposures, blood styrene in CBS is categorized with respect to the 95<sup>th</sup> percentile of NHANES blood styrene levels. In lieu of a clinical cutpoint, we designate these participants as elevated styrene

cases and compare age- and sex-standardized rates of elevated styrene arising from CBS and NHANES. This analysis is summarized with a rate ratio.

### **Predict measured blood styrene levels using NATA and GuLF STUDY data**

We explored associations between identified predictors and blood styrene levels using correlations to inform regression-based methods for predicting blood styrene levels. The reduction in residual variance ( $R^2$ ) is reported for predictive associations.

Because NATA estimates are averages, instead of analyzing ambient styrene concentrations as continuous measures, we designated rank-based exposure assignment using quantiles based on the distribution of NATA estimates across the five-state Gulf region. NATA estimates are added to predictive models to capture the excess contribution of ambient styrene concentration to blood levels, above and beyond the variability explained by individual level predictors collected from questionnaire data.

Reported information from the enrollment, home visit, and biomonitoring questionnaires includes a broad range of potential predictors of styrene exposure. Relationships with continuous styrene exposure are explored using correlation coefficients for continuous covariates, two-sample t-tests for binary covariates, and one-way analysis of variance for categorical covariates.

We use linear least squares regression to predict continuous blood styrene levels. Blood styrene was evaluated for potential log-transformation, and we also used the binary designation relative to the smoking-specific NHANES 95<sup>th</sup> percentile.

A purely predictive approach to model specification is applied to meet the objective of predicting blood styrene levels in Specific Aim 1 [152]. A literature review of

occupational and environmental styrene exposure was conducted to identify potential determinants of styrene blood levels. The joint distribution of each potential predictor with blood styrene is examined to explore potential associations and determine which measure of a given predictor type demonstrates the strongest relationship with the outcome.

We use stepwise regression to select predictors for the final model. First, each of the candidate predictor variables is evaluated in a univariate regression model with measured blood styrene as the dependent variable. Next, variables that achieve statistical significance ( $p < 0.05$ ) will be retained for consideration in multivariate models. We use backward elimination with a retention criteria of  $p < 0.10$  for model specification.



## Specific Aim 2

To extend the predictive modeling in Specific Aim 1, we predict measured blood styrene levels using NEI point source data and spatial indicators in Specific Aim 3. Proximity to and density of point source locations (PSLs) relative to a participant's geocoded home residence were applied for location-based exposure estimates. Distance to sources, density of sources, and source emissions volume within a given circular buffer was used to capture the exposure frequency and intensity. The spatial radius of the buffer was determined based on pollutant dispersion ranges [56] and spatial distribution of participants and PSLs in the study region [153]. As such, multiple buffers are evaluated (0.5 miles, 1 mile, 2 miles, 5 miles, and 10 miles).

Proximity measures using distance as a proxy for exposure to industrial emissions have been used for a wide variety of pollutants at a broad range of spatial and temporal scales [154-161]. Though these measures are often criticized for making crude assumptions about pollutant dispersion behavior and volume [162], their use is recommended in conjunction with an additional marker of exposure, such as NATA estimates [163]. The following *distance based measures* of exposure are considered:

- Euclidean distance to nearest PSL
- Number of PSLs within a given radius
- Inverse distance weighted (IDW) count of point source locations within a given radius of geocoded residence, a continuous exposure metric:

$$IDW\ PSL\ count = \sum_{i=1}^n \frac{1}{d_i}, \text{ where } d_i = \text{distance of the } i\text{th individual PSL}$$

from residence;  $n$  = number of existing PSLs within given radius [164].

Emissions weighted proximity measures (EWPMs) have been used extensively in air pollution epidemiology, particularly for HAPs. Evaluations of spatially based exposure estimation in Texas indicate that EWPM estimation quantifies exposure more accurately than traditional proximity measures, and is a valid alternative to NATA [165, 166]. The following *emissions based measures* of exposure are considered:

- Annual styrene emissions volume within a given radius
- Emissions volume weighted distance to nearest PSL
- Emissions volume weighted IDW PSL count

In addition to distance and emissions based exposure assessments, facility characteristics were evaluated. Facilities were categorized according to SCC and NAICS codes to determine whether specific industries, activities, or source regime combinations are associated with increased emissions or blood styrene levels.

We use a similar modeling approach to that described in Specific Aim 1 to predict continuous (natural log-transformed) blood styrene levels, now selecting predictors from the range of variables describing proximity to point source emissions. Log binomial regression models are used to estimate associations between PSL styrene exposure and prevalence of elevated styrene. We ultimately combine the results of Specific Aims 1 and 2 to assess the combined predictive ability of both exposure approaches. We use backward elimination with a retention criteria of  $p < 0.10$  for model specification, building a combined model from predictors identified in Specific Aims 1 and 2.

### **Specific Aim 3**

For Specific Aim 3, we evaluate the association between environmental styrene exposure and neurologic outcomes:

Estimate associations between ambient styrene and neurologic symptoms

Estimate associations between blood styrene and neurologic symptoms

Estimate associations between styrene exposure and peripheral neurologic function

#### **Estimate associations between ambient styrene and neurologic symptoms**

The principal analysis estimates associations between ambient styrene exposure metrics (NATA estimated concentrations) and self-reported symptoms of neurologic function with respect to the preceding 30 days.

We use log-binomial regression to estimate associations (prevalence ratios, PR, and 95% Confidence Intervals, 95% CI) between ambient styrene and self-reported neurologic symptoms (N=21,962). Outcomes are dichotomized as presence or absence of a given symptom. Separate multivariate regression models are implemented for each symptom.

Due to the likelihood of co-occurring symptoms, we explored the use of latent class analysis to analyze grouped neurologic symptoms. As previously mentioned, seizures, vomiting, and insomnia are excluded from latent class analyses (due to incomplete enumeration). Two neurologic symptom clusters were identified: a central nervous system (CNS) cluster, including dizziness, headache, nausea, sweating, and palpitations; and a peripheral nervous system (PNS) cluster, including tingling and numbness in extremities, blurred vision, and stumbling. In addition to specific individual

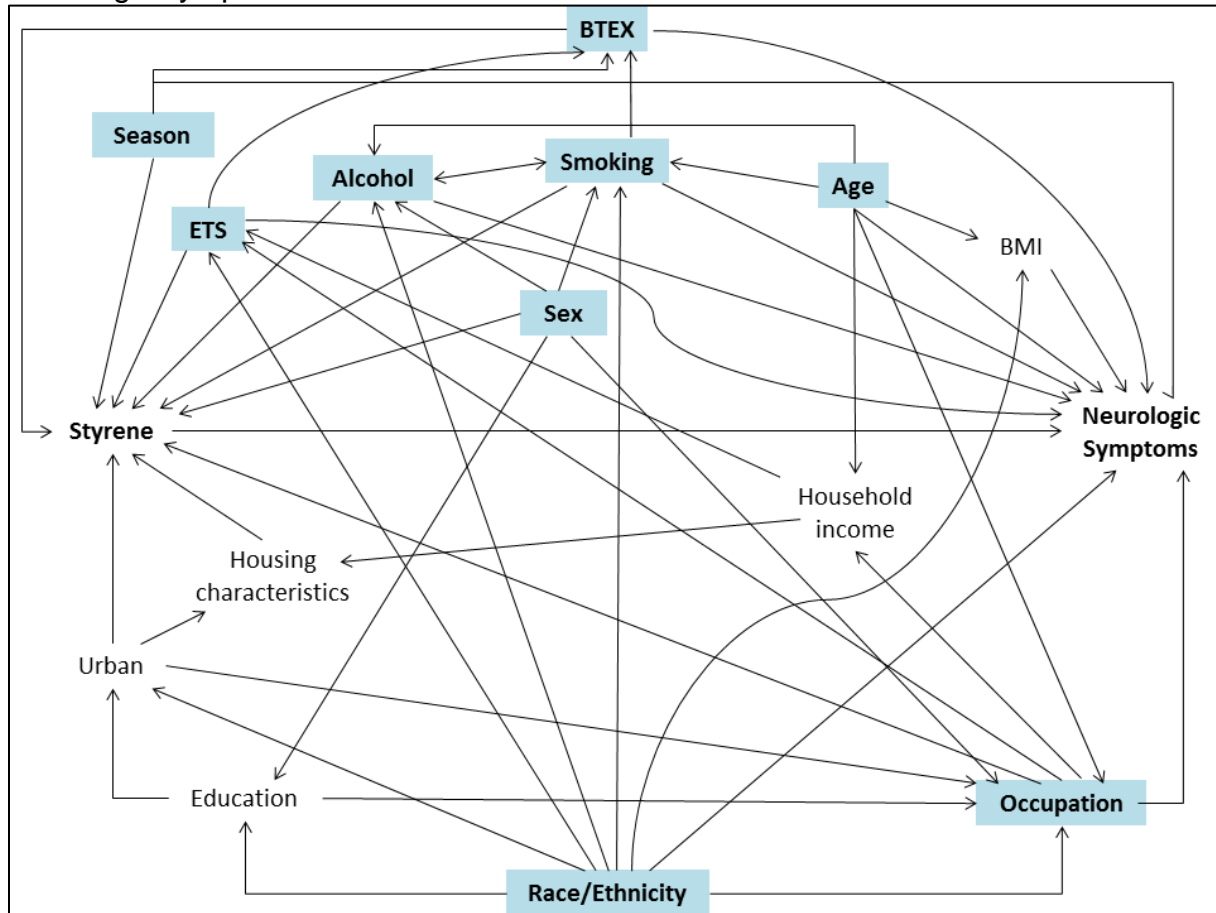
neurologic symptom analyses, we estimate associations between ambient styrene exposure and grouped outcomes: CNS, PNS, and any neurologic symptoms.

Potential confounders were identified based on the existing literature, and a minimally sufficient adjustment set was selected using directed acyclic graph (DAG) analysis of the theoretical relationship between styrene exposure and neurologic impairment (Figure 3). The following covariates are prioritized for inclusion in the causal diagram:

- Demographics: age, sex, race, ethnicity, education, and income
- Lifestyle: smoking, alcohol consumption, BMI, occupation
- Environment: urban residence, population density, BTEX exposure, environmental tobacco smoke

The minimally sufficient set identified using DAG analysis includes: {age, sex, race/ethnicity, season, occupation, alcohol consumption, cigarette smoking, and BTEX coexposure}. Age, sex, and race/ethnicity were all reported at enrollment via CATI. Season refers to season of study enrollment. Occupation is modeled as employment status (employed vs unemployed), due to limitations in available self-reported occupational data. Alcohol consumption and cigarette smoking are modeled as current users compared to all others, based on self-reported information at enrollment. Due to the correlation between BTEX and styrene exposure, as well as potential for adjusting away main exposure effects, BTEX co-exposure adjustment is relegated to sensitivity analyses only. In the analysis of ambient styrene exposure, NATA estimates are used as measures of BTEX co-exposures.

Figure 3. Directed acyclic graph for the relationship between styrene exposure and neurologic symptoms.



Covariates included in the proposed regression model are shown in bold face type (exposure, outcome, and minimally sufficient adjustment set).

### Estimate associations between blood styrene and neurologic symptoms

We evaluate the association between styrene and neurologic function among CBS participants (N=874) using measured blood styrene as the exposure of interest. These analyses are run in parallel with ambient exposure analyses, using the same statistical approach. Because the theoretical basis for the relationship is constant between the two models, the DAG, and minimally sufficient covariate set are the same. Further, to facilitate comparisons between the NATA estimated associations and the blood styrene associations, the models are run with identical adjustment covariates,

with one exception. Blood exposure analyses are additionally adjusted for the duration of time (days) between enrollment, when symptoms were ascertained, and the CBS home visit, when blood styrene was measured. Appropriately, blood BTEX levels are used as co-exposure adjustment factors in these models.

Because outcome ascertainment occurred during the baseline telephone interview, and exposure assessment was subsequently completed at the home visit, we examine the distribution of elapsed time between enrollment and the home visit. The exposure-outcome analysis remains conceptually cross-sectional because styrene exposures are assumed to be typical, low- or medium-level, long-term exposures contributing to neurological effects assessed in the last month. We conduct sensitivity analyses restricting to participants with baseline and home visit dates that are closer in time. 70% of the study population has a lag of six months or less.

### **Estimate associations between styrene exposure and neurologic tests**

We use multivariate linear regression to estimate styrene-associated continuous differences in peripheral neurologic function. Higher values indicate better performance for all continuous outcomes. To achieve this internal consistency, we report the opposite of the raw values for vibrotactile threshold and postural sway speed. For visual contrast sensitivity, we restrict to participants with better than 20/50 visual acuity (assessed during the clinical examination) and adjust for vision correction, comparing styrene-exposed to unexposed participants and evaluating differences in adjusted mean scores at each spatial frequency.

We use log binomial regression to estimate prevalence ratios and corresponding 95% confidence intervals (PR, 95% CI) for single leg stance. Single leg stance is modeled as inability to maintain balance for 30 seconds to preserve a large enough referent group to maintain reasonable precision.

We analyze each exposure type separately, using identical statistical methods. For associations with ambient styrene (N=2,956), exposure is modeled as quartiles, with the lowest quartile designated as the referent group. For analyses of measured blood styrene (n=310), exposure is dichotomized at the 90<sup>th</sup> percentile of the distribution, defining the top 10% of blood measurements as exposed.

Adjustment factors are identical to those reported for symptoms analyses, though we additionally adjust for height when assessing vibrotactile threshold and handgrip strength.

## CHAPTER IV: DETERMINANTS OF STYRENE EXPOSURE (SPECIFIC AIMS 1 & 2)

### A. Introduction

To address community concerns about exposure to potentially harmful oil spill-related chemicals among Gulf coast residents, we measured blood volatile organic compound (VOC) levels in a subset of Gulf Long-Term Follow-up Study (GuLF STUDY) participants 2-3 years after the *Deepwater Horizon (DWH)* oil spill. We previously reported that blood levels of specific oil-related VOCs such as benzene, toluene, ethylbenzene and xylenes were similar to those found in the National Health and Nutrition Examination Survey (NHANES) (Werder EJ, *in press*). Among other VOCs included in the CDC-based test panel, only styrene was substantially elevated. We further investigated styrene levels due to increased exposure opportunity in the region.

Styrene, an industrial hydrocarbon and hazardous air pollutant, is an established neurotoxicant at occupational levels [1, 5, 6], that has not been studied at environmental levels experienced by the general population. Styrene is used in plastics, fiberglass, rubber, and resins to manufacture consumer products and commercial and residential building materials. Manufactured styrene products include insulation, fiberglass boats, automotive parts, car tires, Styrofoam, and plastic drinking glasses [1]. The U.S. produces over 12 billion pounds of styrene annually, with atmospheric emissions of 28 million pounds per year [1, 19]. Environmental release of styrene from the manufacture, use, and disposal of styrene-based products occurs primarily through the air [18].



Emissions from industrial activities and motor vehicle exhaust are the primary sources of styrene in outdoor air. Ambient measurements of styrene typically show airborne concentrations less than 1 part per billion by volume (ppb). Rural or suburban air generally contains lower concentrations of styrene than urban air [22], which is attributed to increased motor vehicle emissions in urban areas [52]. Elevated ambient styrene concentrations are observed near styrene emitting industries, suggesting that individuals living near manufacturing or processing facilities may experience increased styrene exposure [1]. Ambient styrene, breaks down in the atmosphere within 1 to 2 days [1] and the half-life of styrene in blood is approximately 13 hours [5].

Indoor air styrene levels result primarily from tobacco smoke, off-gassing of building materials and consumer products, and emissions from photocopiers and laser printers [1]. Smoking is the single most important individual predictor of human exposure to styrene [1, 20, 51, 59, 60, 64, 84, 85]. Exposure to environmental tobacco smoke is also associated with higher styrene exposure, particularly for non-smokers living with a smoker [59, 85]. Typically, indoor air contains higher styrene levels than outdoor air [51].

Inhalation of contaminated air is the principal route of styrene exposure for the general population [1], and the main source of concern for health effects due to styrene toxicity [24]. Although ingestion and dermal exposure to styrene can occur in occupational settings, inhalation accounts for over 90% of styrene exposure in the general population [6, 25]. The highest styrene exposures generally occur in the workplace, where styrene-exposed workers have blood levels that are 25 times higher on average than those in the general population [5, 7, 36]. Occupational exposure to

airborne styrene has been well-characterized among workers in boat building and reinforced plastics manufacturing [10, 13, 14, 25, 48-50].

The Gulf States are home to many styrene-emitting industries and over half of all U.S. styrene production [1, 4]. In addition to a prolific petrochemical industry, the Gulf region is home to many industrial and manufacturing facilities that use and emit styrene in the production of plastics, rubber, and fiberglass. This geographic clustering of industries potentially exposes Gulf residents to a disproportionately high intensity of environmental styrene emissions.

## **B. Methods**

### **Study Design and Participants**

Approximately 2-3 years after the April 2010 *Deepwater Horizon (DWH)* disaster and oil spill, we took advantage of the ongoing enrollment of participants in the GuLF STUDY to recruit individuals living in the Gulf region for a study of current blood levels of VOCs. Because styrene is rapidly cleared from the body and blood measurements were obtained 2-3 years after the oil spill, these levels represent exposures occurring around the time of sample collection (i.e., they are not due to oil spill cleanup work). A total of 994 individuals provided blood specimen sufficient for quantification of styrene levels as part of their participation in the Chemical Biomonitoring Study (CBS) to measure current blood VOC chemical levels.

In concert with ongoing home visits for the GuLF STUDY we enrolled participants for the CBS between September 2012 and March 2013. The GuLF STUDY is a prospective cohort of adults (ages 21 and older) who participated in oil spill response

activities and others who received safety training, but were not hired following the *DWH* disaster. A detailed description of this study is available elsewhere [167]. Eligible participants were 11 193 English- or Spanish-speaking individuals who lived in Florida, Alabama, Mississippi, Louisiana, or eastern Texas and participated in a home visit examination. CBS participants were selected from among GuLF STUDY participants whose home exams were scheduled in the later months of study enrollment and participation involved providing an extra blood sample for measuring styrene and other compounds and completing a questionnaire about usual and past 24-hour exposure opportunities. Study personnel geocoded participants' residential locations using handheld global positioning system devices. We initially oversampled nonsmokers and women, but because of timing of the parent study, we ultimately invited all remaining eligible participants to participate. Of the 994 individuals who provided blood samples of sufficient quantity and quality to measure styrene levels [168-170], 935 also had both a measurement for the tobacco smoking biomarker 2,5-dimethylfuran (2,5-DMF) and provided self-reported smoking information. These 935 participants were included in analyses comparing blood VOC levels between the CBS and the NHANES 2005-2008 [171, 172].

We restricted the remaining analyses to participants who had complete information on all modeled predictors (n=667). We excluded participants who did not complete the questionnaire on recent exposure opportunities (n=125) or were missing data on demographic factors (n=142).

Participants provided written consent, and the Institutional Review Board of the National Institute of Environmental Health Sciences approved this study.

## **Exposure Monitoring Questionnaire**

We collected demographic, socioeconomic, occupational, lifestyle, and health information during the GuLF STUDY enrollment and home visit interviews. CBS participants also answered questions about potential contributors to blood styrene levels, including residential building characteristics, self-reported proximity to industrial operations and waste sites (i.e., participants were asked to indicate whether they lived within a half mile of each of the following: major highways, a boatyard, docks, an oil refinery, a petroleum storage or transfer facility, a gas station, a factory, a power plant, a hazardous waste or Superfund site, and a landfill), personal chemical exposures, perceived air quality, drinking and bathing water source, smoking and tobacco use, and hobbies and activities, including exposure opportunities in the past 24 hours (e.g. refueling vehicles or lawn equipment), using an adapted version of the Centers for Disease Control and Prevention (CDC) NHANES 2007-2008 questionnaire [171] and US Environmental Protection Agency Detroit Exposure and Aerosol Research Study (DEARS) survey [173].

## **National Emissions Inventory**

Point sources of styrene emissions were identified using the 2011 National Emissions Inventory Version 2 (NEI), the United States Environmental Protection Agency's (EPA's) latest comprehensive database of annual criteria, precursor, and hazardous air pollutant emissions [17]. State, local, and tribal air agencies report emissions sources, which are then augmented with information from the Toxics Release Inventory, the Acid Rain Program, and EPA's regulatory air toxics data.

We abstracted all records of reported styrene emissions from the 2011 NEI point source database and mapped participants' home geocodes onto the locations of the NEI point sources to calculate the distance between each participant's residence and proximal point sources. We assigned exposure to point sources of styrene emissions based on number of sources, linear distance to point source locations, and volume of emissions at one-half, one, two, five, and ten mile buffers surrounding participants' homes.

### **National Air Toxics Assessment**

The EPA's 2011 National-scale Air Toxics Assessment (NATA) evaluates 180 air toxics across the United States using emissions inventories, dispersion modeling, photochemical modeling, exposure modeling, and toxicity analyses [16, 174]. NATA uses dispersion modeling to generate annual average ambient air toxic concentrations for each U.S. census tract. The model predicts annual average census tract concentrations for each major source type (point, nonpoint, mobile), which are then summed to a total concentration value for all source types combined. Styrene concentrations in NATA 2011 arise from all three major source types.

We employed NATA styrene estimates as indicators of typical, long-term environmental exposure by mapping each participant's geocoded home location to a 2010 U.S. census tract, and applying the annual average total and source-partitioned concentrations for that census tract to the participant.

## **Blood collection and blood VOC measurements**

Glass blood collection tubes containing potassium oxalate and sodium fluoride anticoagulant were used to collect 10 mL of blood for styrene measurement. Blood samples were collected using tubes and stoppers that had been pre-treated by the CDC laboratory to remove VOC residues to minimize pre-collection contamination [175, 176]. Samples were stored in a 4°C refrigerator prior to being shipped overnight on cold packs in biweekly batches to the Centers for Disease Control and Prevention in Atlanta, Georgia for analysis of VOCs. This laboratory conducts all NHANES VOC analyses. Analysis of styrene followed the standard CDC procedures for NHANES samples, using equilibrium headspace solid-phase micro-extraction with benchtop gas chromatography/mass spectrometry [168, 170], allowing direct comparisons between measurements in CBS and NHANES. 3 mL of blood was required per analysis.

We measured 2,5-dimethylfuran (2,5-DMF), a VOC used as a smoking biomarker with comparable sensitivity and specificity to serum cotinine (a well-validated nicotine biomarker) [177]. Blood 2,5-DMF concentration of 0.014 ng/mL has been established as a threshold for distinguishing between current daily smokers ( $\geq 0.014$  ng/mL) and nonsmokers ( $< 0.014$  ng/mL) [177, 178], with the latter comprising infrequent smokers whose blood styrene levels have returned to that of nonsmokers. Unless otherwise specified, we use this definition to identify smokers and nonsmokers throughout all analyses.

## Statistical Analysis

We compared the distributions of blood styrene levels measured in CBS participants (n=935) to those observed in NHANES participants ages 21 and older who had blood styrene measured during the 2005-2006 and 2007-2008 NHANES cycles (N=3,958). All comparisons between NHANES and CBS were stratified by the 2,5-DMF threshold for smoking status (0.014 ng/mL). For comparisons to NHANES, we imputed blood styrene concentrations below the limit of detection (LOD) as the LOD divided by the square root of two [179], as is done in NHANES. For all other statistical analyses, we used all measured blood styrene values, including the actual values below the LOD [180].

We also calculated the smoking-specific age- and sex-standardized prevalence ratio of CBS participants with blood styrene levels above the NHANES 95<sup>th</sup> percentile standardized to the CBS sample. We presented this standardization approach without applying NHANES sampling weights, but also conducted parallel analyses using NHANES sampling weights to verify that the weighting approach didn't influence results. Styrene concentrations were approximately log-normally distributed, so we used natural logarithmically-transformed concentrations in continuous analyses.

For regression analyses, we further restricted to participants who completed the exposure monitoring questionnaire (n=810) and had complete covariate information for demographic factors, potential predictors, and ambient exposure metrics (n=667). We selected predictors *a priori* based on previous literature [59, 181-186], with residential building characteristics, lifestyle and behaviors, recreational and occupational activities, and relevant recent exposures as candidate predictors. We additionally considered

ambient styrene predictors, including NATA total and source-partitioned (point, nonpoint, and mobile) concentrations as well as NEI point source emissions of styrene. Metrics of emissions included binary indicators (presence or absence of sources), number of sources, and distance- and volume-weighted intensity of sources surrounding participants' homes. We used analysis of variance and t-tests to prioritize candidates based on the strength and statistical significance of their unadjusted relationship with blood styrene levels.

We implemented a predictive modeling approach using least squares regression aimed at maximizing the model adjusted  $R^2$ , and retained covariates with p-values < 0.10. We chose this approach because many sources of styrene exposure were rare in this population. We maintained a statistical significance threshold of  $\alpha=0.05$ , and report the change in log-styrene concentration ( $\beta$  coefficient) attributed to each predictor, and its associated 95% confidence interval and p-value.

We also used multivariable regression to estimate prevalence ratios and corresponding 95% confidence intervals (PR, 95% CI) for a blood styrene measurement exceeding the smoking-specific NHANES 95<sup>th</sup> percentile. Due to model convergence problems for the log-binomial model, all analyses were completed using a modified log-binomial approach with a Poisson distribution [187]. The same predictive modeling approach described for the analysis of continuous styrene was used to specify the modified Poisson model.

Due to concerns about varying data reporting and quality of NATA estimates between states, we evaluated agreement between NATA modeled ambient styrene concentrations and observed styrene concentrations from EPA Ambient Monitoring



Archive (AMA) monitors in the study region. We compared annual average monitored concentrations to corresponding census tract NATA estimates for each state. Lacking sufficient sample size to conduct individual state regression analyses, we conducted sensitivity analyses excluding one state at a time to evaluate the influence of state reporting differences on the association between NATA exposure data and elevated blood styrene levels.

To more efficiently examine ambient predictors of elevated blood styrene (i.e., NATA and NEI exposures), we identified a subgroup with no tobacco smoke-related styrene exposure by restricting to participants with blood 2,5-DMF < 0.014 ng/mL and removing an additional 97 individuals who reported any active or passive tobacco smoke exposure in the past 24 hours (n=300). We further restricted to participants living in states where NATA best reflected observed AMA concentrations (n=224), and removed individuals with spring and summer blood draws to account for seasonal differences in ambient styrene, resulting in a sample of 195 participants. We then used the same modified Poisson model to estimate associations between ambient exposures and blood styrene exceeding the NHANES 95<sup>th</sup> percentile in this subgroup.

All statistical analyses were conducted in SAS 9.4 (Cary, NC, USA) and spatial analyses were completed in Esri ArcGIS Desktop 10.3 (Redlands, CA, USA).

### **C. Results**

Blood styrene levels for these CBS participants (n=935) were two to three times higher than those reported in NHANES (n=3,958) (Table 3) for both smokers and nonsmokers, but considerably lower than levels typically observed among occupationally exposed populations. Among nonsmokers, detection rates were

considerably higher in CBS (61.9%) than NHANES (23.7%). Among smokers, rates were more comparable between study populations (CBS, 99.2%; NHANES, 96.1%). The distribution of blood styrene in CBS is right-skewed, regardless of smoking status. Nonsmokers in CBS were almost six times as likely to have elevated blood styrene (blood measurement above the NHANES 95<sup>th</sup> percentile), and smokers in CBS were more than four times as likely to have elevated blood styrene, as their NHANES counterparts (Figure 4). The apparent second peak in the blood styrene distributions above the NHANES 95<sup>th</sup> percentile for both smokers and non-smokers in CBS suggests that an elevated styrene measurement may indicate membership in a subgroup with a higher mean exposure distribution.

Overall, two-thirds of CBS participants included in predictor models (n=667) are younger than 50, three-fourths are male, half are white, and three-fourths are overweight or obese (Table 4). Most participated in oil spill cleanup work (85.8%), and over half are unemployed. Approximately 40% of modeled CBS participants had blood 2,5-dimethylfuran levels characteristic of smoking. Smokers tended to be younger, less educated, more likely to be nonwhite, and have lower body mass index. They were also more likely than nonsmokers to report lower income and unemployment.

We identified several predictors of blood styrene levels (Figure 5), though the overall model adjusted R<sup>2</sup> was 0.19. Continuous log-transformed blood 2,5-dimethylfuran level was a strong predictor of increasing blood styrene level ( $\beta$ , 0.42 log ng/mL; 95% CI: 0.34, 0.51), demonstrating the importance of smoking as a styrene exposure source (Table 5). Living in a home painted in the past six months (n=82;  $\beta$ , -0.46 log ng/mL; 95% CI: -0.75, -0.17) was associated with lower styrene levels, while

concrete/cinderblock (n=169;  $\beta$ , 0.37; 95% CI: 0.11, 0.64) and wood (n=265;  $\beta$ , 0.35; 95% CI: 0.13, 0.57), home exteriors predicted higher blood styrene levels. Other significant predictors of increasing styrene included boating in the Gulf in the past 24 hours (n=10;  $\beta$ , 1.10; 95% CI: 0.31, 1.89), spending at least three hours in motor vehicles in the past 24 hours (n=122;  $\beta$ , 0.34; 95% CI: 0.09, 0.59), living in a mobile home, RV, or boat (n=99;  $\beta$ , 0.35; 95% CI: 0.07, 0.63), and reporting recreational fishing (n=330;  $\beta$ , 0.20; 95% CI: 0.0002, 0.39). Being employed (n=374;  $\beta$ = 0.19; 95% CI: -0.01, 0.38) was a positive predictor of blood styrene, of borderline significance. Blood draws in fall or winter (n=597;  $\beta$ , -0.41; 95% CI: -0.73, -0.10) were associated with lower blood styrene levels, compared to spring and summer blood draws. Ambient nonpoint styrene concentrations were weakly and non-significantly predictive of increasing blood styrene levels, with the strongest association in the second quartile. State of residence was non-significantly associated with blood styrene levels, with Florida residents (n=165) having the highest blood styrene levels ( $\beta$ , 0.27; 95% CI: -0.07, 0.61). Predictors of elevated blood styrene in the overall sample were similar in magnitude and direction to the observed predictors of continuous blood styrene (Figure 6).

Predictors of elevated blood styrene (above the NHANES 95<sup>th</sup> percentile) among participants unexposed to tobacco smoke (active or passive), with fall or winter blood draw, and not living in Alabama included ambient nonpoint styrene concentration and Florida residence (Figure 7). This subgroup was identified to more efficiently assess ambient, as opposed to personal, predictors of styrene. We excluded Alabama based on results of a sensitivity analysis examining associations between NATA estimated concentrations and AMA observed concentrations by state (Appendix 2). Using the

limited data available, we determined that Alabama demonstrated appreciably less agreement with observed concentrations than the four remaining states. In this subgroup analysis, we observed a weak increasing exposure-response relationship across quartiles of ambient nonpoint styrene concentration, where participants in the highest exposure group were twice as likely to have elevated blood styrene as those in the lowest group (PR, 2.1; 95% CI: 1.0, 4.4). Florida residents were twice as likely to have elevated blood styrene as their counterparts from Mississippi (PR, 2.0; 95% CI: 1.1, 3.7). Exposure to point source emissions of styrene was not associated with elevated blood styrene levels for any of the exposure metrics or spatial scales we examined.

#### **D. Discussion**

We conducted this study to characterize blood styrene levels among Gulf coast residents and identify determinants of styrene exposure. Blood styrene levels among CBS participants were substantially elevated compared to NHANES, particularly in the upper tail of the distribution. These levels, while higher than NHANES, are orders of magnitude lower than occupational exposure levels. Participants living in Florida had the highest blood styrene levels. Personal, as well as environmental factors, predicted blood styrene levels in the overall CBS sample. In a subgroup identified to facilitate examination of environmental factors, ambient styrene from nonpoint sources was associated with elevated blood styrene with a suggestive exposure-response relationship. However, proximity to industrial styrene emissions from point sources was not related to blood styrene.

Although several different environmental, behavioral, and social factors have been identified as determinants of styrene exposure, these factors typically explain less than 25% of the variance in measured styrene exposure [52, 59]. In our study, smoking, spending time in boats or vehicles in the past 24 hours, recreational fishing, being employed, and living in mobile housing were positively associated with blood styrene levels. Living in a home painted in the past six months and fall/winter blood draws were inversely associated with blood styrene levels.

We observed a highly significant, precise, positive association between the biomarker for smoking (2,5-dimethylfuran) and blood styrene. Previous studies indicate that smokers have blood styrene levels approximately fourfold higher than those of nonsmokers [1, 51, 59-65]. Although cigarettes are considered the dominant source of styrene exposure among smokers, environmental tobacco smoke contributes only about 8% of nonsmokers' exposure [20].

Spending at least three hours in a vehicle or any time in a boat in the Gulf of Mexico in the past 24 hours were both associated with increasing styrene levels. Likewise, recreational fishing was a predictor of styrene exposure. These predictors likely confer increased styrene exposure from combustion engine emissions, an established source of outdoor styrene exposure [20, 52, 75, 87].

The association between employment and increasing blood styrene suggests that sources outside the home could be driving higher exposures in non-smokers [75]. We conducted a preliminary analysis of reported occupation, industry, and main job activities to ascertain styrene-related occupational exposure opportunities. We

determined that such opportunities were too rare in our population (<0.1%) to account directly for the association between employment status and blood styrene.

Participants who reported living in mobile housing had higher blood styrene levels. This association may reflect housing materials with more off-gassing than other types of residences, or lower air exchange rates resulting in higher indoor styrene levels [86]. Mobile housing is likely to be smaller than apartments, townhouses, and detached homes, with fewer rooms and windows. Previously, smaller house size and lower number of windows have been associated with increased styrene exposure [75].

Blood styrene levels are fairly consistent within season, though monitoring suggests significantly lower styrene levels in the spring compared to all other seasons [56]. We observed lower styrene levels in fall and winter compared to spring and summer, however the seasonal distribution of blood collection (90% in fall or winter) precluded seasonally-stratified analyses and further exploration into this relationship. Previous research suggests that individuals living near styrene manufacturing or processing facilities may experience increased exposure from point source emissions, totaling 47.3 million pounds annually in the U.S. [1]. Residential proximity to styrene emitting industrial facilities has been shown to confer up to 15 ppb of annual average styrene exposure [26] with elevated concentrations detected up to 10 kilometers away [55]. In our analyses, however, styrene levels varied independently of residential proximity to point source emissions, with no relationship to volume, intensity, distance, or type of emission source at a range of buffers from 0.5 miles to 10 miles. Similarly, NATA estimated concentrations attributable to point sources were not associated with blood styrene levels. Our measures of exposure to point source emissions may be

insufficient to capture episodic exposure scenarios, as both data sources rely on annual reporting. These measures fail to reflect seasonality or temporal variability that may influence prediction of a single blood draw. Because national-scale ambient styrene monitoring is limited, with poor spatial and temporal coverage, we were unable to evaluate monitored styrene concentrations in association with contemporaneous blood styrene levels.

We evaluated total NATA concentrations, as well as source-partitioned values for each major source type. Total, point, and mobile concentrations were not associated with styrene levels in the full study population. However, when we looked in the subgroup identified to better examine environmental sources of elevated blood styrene, NATA nonpoint styrene concentration showed a positive exposure response relationship. This association only emerged among nonsmokers with no reported environmental tobacco smoke exposure, who had fall/winter blood draws, and did not live in Alabama. We selected this group to reduce other major sources of styrene variability and measurement error so that we could better assess the relationship between ambient and blood styrene. Improving the signal to noise ratio in this way did allow us to detect an association that was not apparent in the main analysis.

Nonpoint sources are smaller sources or sources related to residential activity (residential wood combustion, consumer and commercial solvent usage, etc.), which are inventoried at the county level, and subsequently allocated to census tracts. These types of styrene exposure sources may demonstrate more temporal consistency within seasons than point source emissions, and therefore be more relevant predictors of spot blood styrene levels than sources which vary episodically. Reporting for nonpoint

sources may also be more reliable than other major source types, so that modeled concentrations are a more accurate representation of nonpoint exposures than other source types.

The primary strength of our study is the examination of both indoor and outdoor predictors of internal burden of blood styrene in a population with elevated blood styrene levels. While other studies have typically prioritized either indoor or outdoor styrene sources, our study includes detailed indoor exposure information, as well as two different assessments of outdoor styrene levels and sources.

We also used an established biomarker of internal styrene body burden, with a detailed, previously validated questionnaire capturing the relevant timing for the blood measurement [173]. This recall period, 24 hours, was sufficiently short to minimize the risk of recall bias and matched the styrene elimination half-life of approximately 13 hours. We ascertained smoking using a validated biomarker, and supplemented with self-reported information on environmental tobacco smoke in sensitivity analyses. Blood styrene and 2,5-dimethylfuran levels in both CBS and NHANES were analyzed in the same laboratory, using the same methods, which permit quantification of general population exposure levels [168]. Geocoding participants' residential locations allowed for a variety of flexible spatial analyses incorporating distance decay with point source emissions. Finally, our study was carried out in an understudied population that has been frequently exposed to multiple natural and man-made disasters, and lives in a region with enhanced industrial styrene exposure opportunity.

Repeated biomarker measurements may provide a more reliable estimate of usual exposure than the single blood specimen obtained in our study, particularly



because of the rapid elimination of styrene from the body. Given the half-life of styrene in blood, blood levels reflect only exposures experienced within the past 12-24 hours. Occupational data were reported at enrollment and potentially not reflective of the 24-hour exposure window preceding the blood draw. Despite this limitation, it is unlikely that occupational styrene exposure is influencing blood styrene levels in this study population because styrene-related occupations tend to be highly specific and were reported rarely among CBS participants. Candidate occupations we considered included: working in a fiberglass reinforced plastic or cultured marble factory, fiberglass boat building or repair, manufacturing styrene resin, polymer, rubber, or styrene-butadiene rubber tires, manufacturing fiberglass wind turbines, and relining sewer lines with styrene-based resins pipe.

Reporting to the NEI database is voluntary and designed for regulatory purposes. As such, the quality of the data and availability of information for our intended investigation may not be ideal. These data are limited to annual aggregate values, and lack any temporal specificity. Similarly, the NATA estimates are annual averages derived from voluntarily reported inputs, the protocols for which vary between states. Although imperfect, in the absence of sufficient monitoring data, these data sources provide the best available outdoor exposure information for a study of our scope and purpose.

The modestly elevated blood styrene levels among CBS participants created a unique opportunity to assess a range of environmental styrene exposures. Although we were only able to account for approximately 20% of the variability in styrene exposure, we identified several personal predictors of blood styrene, which were largely consistent

with what has been published previously. Despite intense styrene-related industrial activity across the study region, we did not observe any relationship between proximity to point source styrene emissions and blood styrene. Rather, our results suggest that, at least among individuals without appreciable tobacco smoke exposure, exposure from nonpoint sources may be an important predictor of blood styrene levels. Future research in this area would benefit from repeated measures of blood styrene, as well as temporally specified environmental styrene sources, whether through monitoring or improved modeling.

#### **E. Conclusions**

The slightly elevated blood styrene levels among CBS participants created a unique opportunity to assess a range of environmental styrene exposures. Although we were only able to account for approximately 20% of the variability in styrene exposure, we identified several personal predictors of blood styrene, which were largely consistent with what has been published previously. Despite intense styrene-related industrial activity across the study region, we did not observe any relationship between proximity to point source styrene emissions and blood styrene in any analyses. Rather, exposure from nonpoint sources emerged as a potential predictor of blood styrene in a subgroup analysis. Future research in this area should incorporate repeated measures of blood styrene, as well as temporally specified environmental styrene sources, whether through monitoring or improved modeling.

## F. Tables and Figures

Table 3. Blood styrene levels in the CBS (n=935) and NHANES (2005-2008) (n=3,958).

	Nonsmokers <sup>1</sup>		Smokers <sup>2</sup>	
	CBS	NHANES	CBS	NHANES
N	551	3,137	384	821
Percent detect	61.9	23.7	99.2	96.1
Mean	0.26	0.03	0.34	0.11
Geometric mean	0.07	0.03	0.15	0.07
25th percentile	< LOD	< LOD	0.07	0.06
Median	0.04	< LOD	0.12	0.09
75th percentile	0.08	< LOD	0.28	0.13
95th percentile	1.67	0.06	1.62	0.23
Maximum	4.81	4.10	4.58	0.78

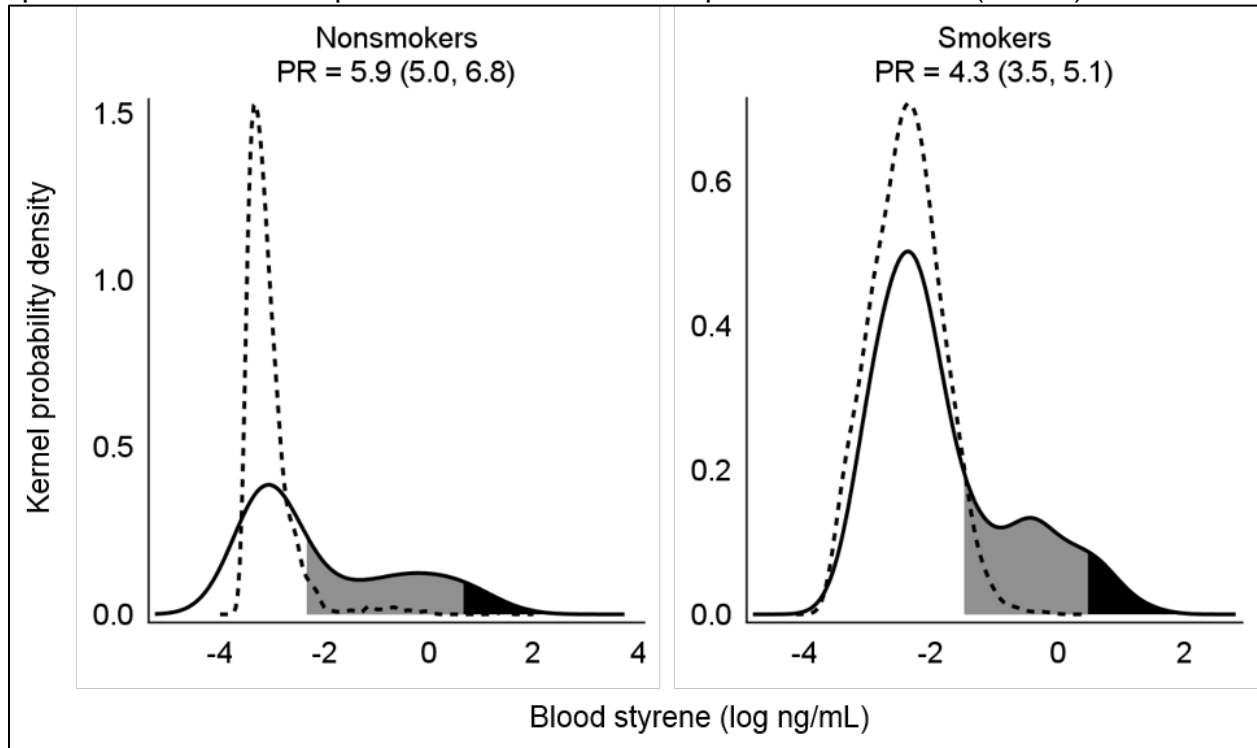
Blood styrene concentration measured in ng/mL. Limit of detection (LOD) = 0.03 ng/mL.

Values below the LOD are imputed as  $\text{LOD} / \sqrt{2} = 0.02$  ng/mL.

<sup>1</sup> Nonsmokers defined as having blood 2,5-dimethylfuran > 0.014 ng/mL.

<sup>2</sup> Smokers defined as having blood 2,5-dimethylfuran concentration  $\geq 0.014$  ng/mL.

Figure 4. Prevalence ratio of blood styrene concentrations exceeding the smoking-specific NHANES 95th percentile in the CBS compared to NHANES (n=935).



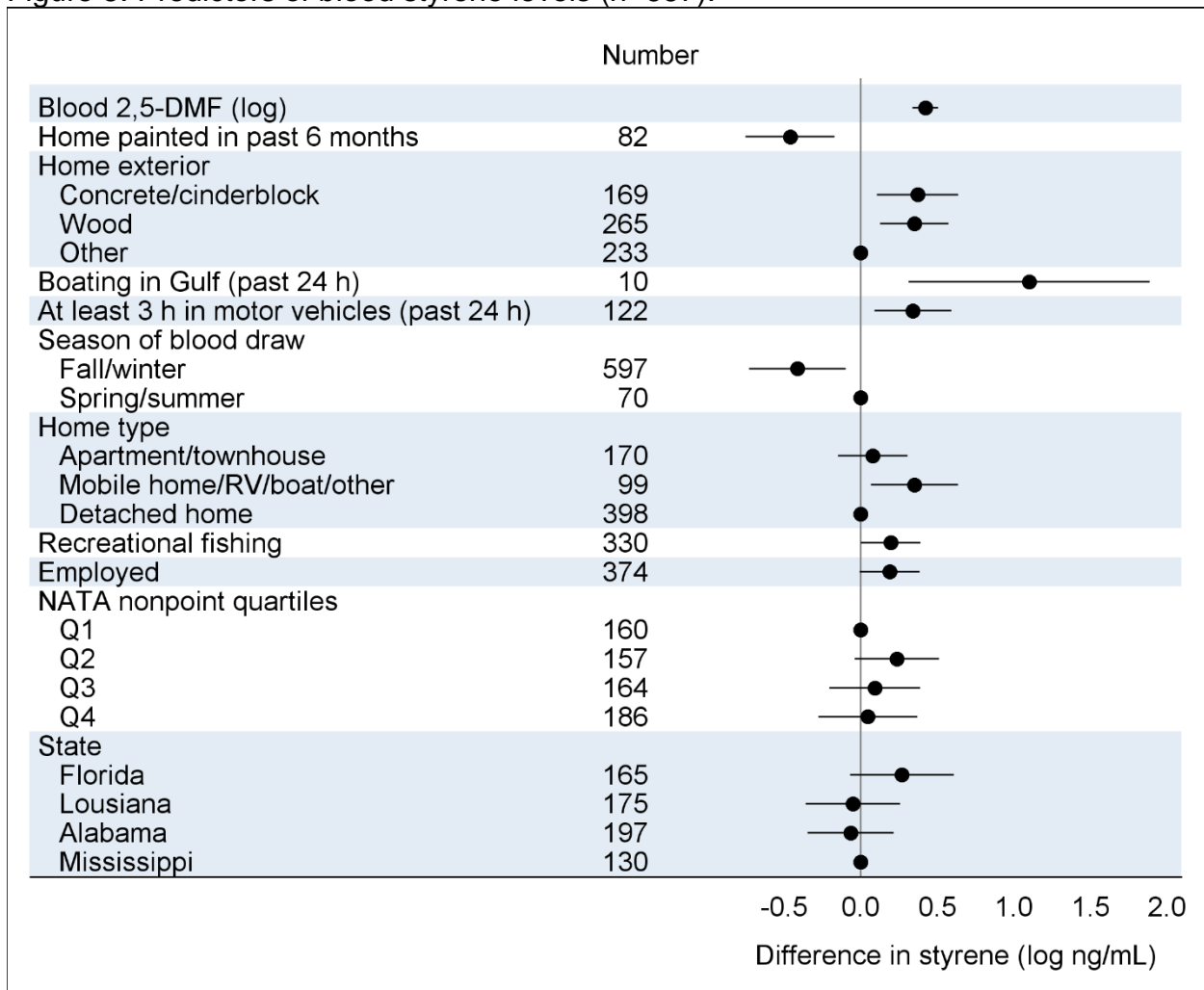
Solid line indicates BTEX study; black shading indicates BTEX study 95<sup>th</sup> percentile. Dashed line indicates NHANES; grey shading indicates NHANES 95<sup>th</sup> percentile. NHANES 95<sup>th</sup> percentile: nonsmokers, 0.06 ng/mL; smokers, 0.23 ng/mL. PR, prevalence ratio (95% confidence interval).

Table 4. Characteristics of participants with blood styrene measurements in the CBS (n=667).

	Overall (n=667)		Smokers <sup>1</sup> (n=270)		Nonsmokers <sup>1</sup> (n=397)	
	N	%	N	%	N	%
Age (years)						
< 30	123	18.4	59	21.9	64	16.1
30 - 50	325	48.7	138	51.1	187	47.1
> 50	219	32.8	73	27.0	146	36.8
Body Mass Index (kg/m <sup>2</sup> )						
< 25	159	23.8	89	33.0	70	17.6
25 - 30	200	30.0	75	27.8	125	31.5
> 30	308	46.2	106	39.3	202	50.9
Education						
< High school grad.	128	19.2	66	24.4	62	15.6
High school grad.	249	37.3	109	40.4	140	35.3
> High school grad.	290	43.5	95	35.2	195	49.1
Race						
Black	277	41.5	133	49.3	144	36.3
White	336	50.4	118	43.7	218	54.9
Other	54	8.1	19	7.0	35	8.9
Sex						
Female	164	24.6	57	21.1	107	27.0
Male	503	75.4	213	78.9	290	73.0
Timing of blood draw						
Fall/Winter	597	89.5	244	90.4	353	88.9
Spring/summer	70	10.5	26	9.6	44	11.1
Work status						
Employed	374	43.9	116	43.0	258	65.0
Unemployed	293	56.1	154	57.0	139	35.0
Oil spill clean-up work						
≥ 1 day	572	85.8	242	89.6	330	83.1
None	95	14.2	28	10.4	67	16.9
Annual income						
< \$20,000	275	41.2	140	51.9	135	34.0
\$ 20,000 - \$50,000	230	34.5	93	34.4	137	34.5
> \$50,000	162	24.3	37	13.7	125	31.5

<sup>1</sup> Smokers defined as having blood 2,5-dimethylfuran concentration  $\geq 0.014$  ng/mL; nonsmokers, blood 2,5-dimethylfuran  $> 0.014$  ng/mL.

Figure 5. Predictors of blood styrene levels (n=667).



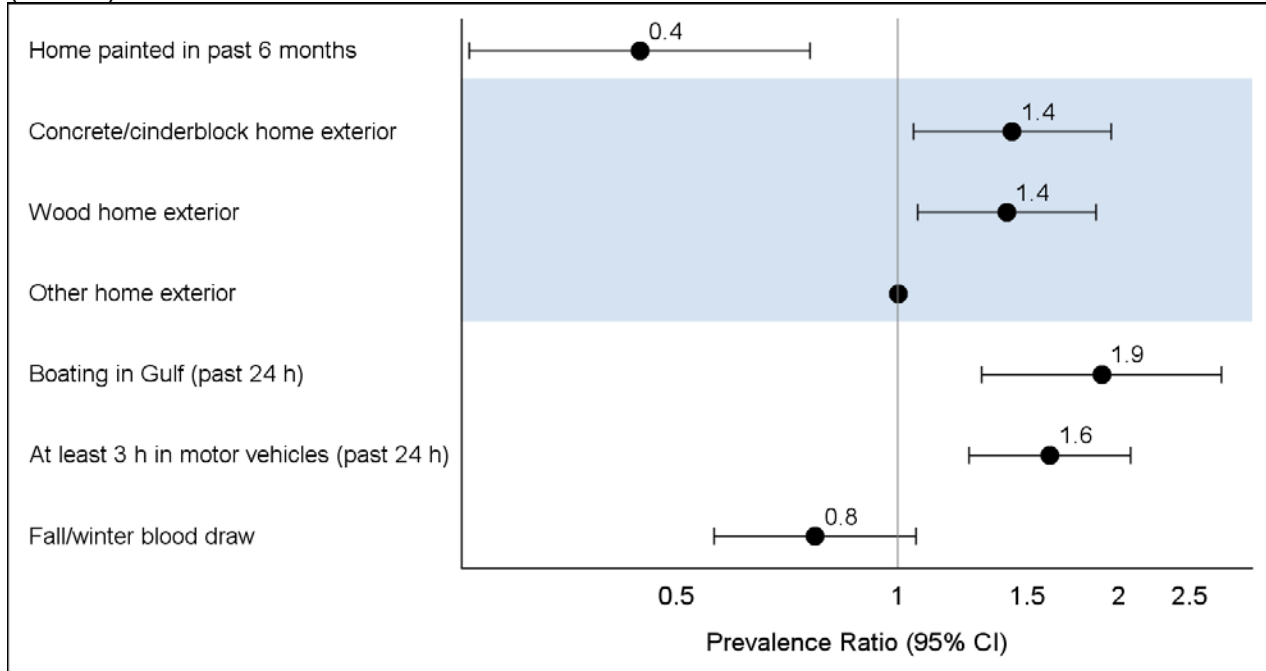
Blood 2,5-DMF (log), log transformed blood 2,5-dimethylfuran concentration.  
 NATA nonpoint, National Air Toxics Assessment 2011 annual average census tract ambient styrene concentration attributable to nonpoint sources. Q1, First quartile; Q2, Second quartile; Q3, Third quartile; Q4, Fourth quartile.  
 State, participant's state of residence.

Table 5. Predictors of blood styrene levels (n=667).

Predictor	N	$\beta$ (95% CI)	p-value
Blood 2,5-DMF (log)		0.42 (0.34, 0.51)	<.0001
Painted home in past 6 months	82	-0.46 (-0.75, -0.17)	0.002
Home exterior			
Concrete/cinderblock	169	0.37 (0.11, 0.64)	0.006
Wood	265	0.35 (0.13, 0.57)	0.002
Other	233		
Boating in Gulf in past 24 hours	10	1.10 (0.31, 1.89)	0.006
At least 3 hours in motor vehicles in past 24 hours	122	0.34 (0.09, 0.59)	0.008
Season of blood draw			
Fall/winter	597	-0.41 (-0.73, -0.10)	0.01
Spring/summer	70	Ref	
Home type			
Apartment/townhouse	170	0.08 (-0.15, 0.31)	0.49
Mobile home/RV/boat/other	99	0.35 (0.07, 0.63)	0.02
Detached home	398	Ref	
Recreational fishing	330	0.20 (0.0002, 0.39)	0.05
Employed	374	0.19 (-0.01, 0.38)	0.06
Ambient nonpoint styrene, quartiles			
1 <sup>st</sup> quartile	160	Ref	
2 <sup>nd</sup> quartile	157	0.24 (-0.04, 0.51)	0.09
3 <sup>rd</sup> quartile	164	0.09 (-0.21, 0.39)	0.55
4 <sup>th</sup> quartile	186	0.05 (-0.28, 0.37)	0.78
State			
Florida	165	0.27 (-0.07, 0.61)	0.12
Louisiana	175	-0.05 (-0.36, 0.26)	0.74
Alabama	197	-0.07 (-0.35, 0.21)	0.64
Mississippi	130	Ref	

2,5-DMF (log), log transformed blood 2,5-dimethylfuran concentration.

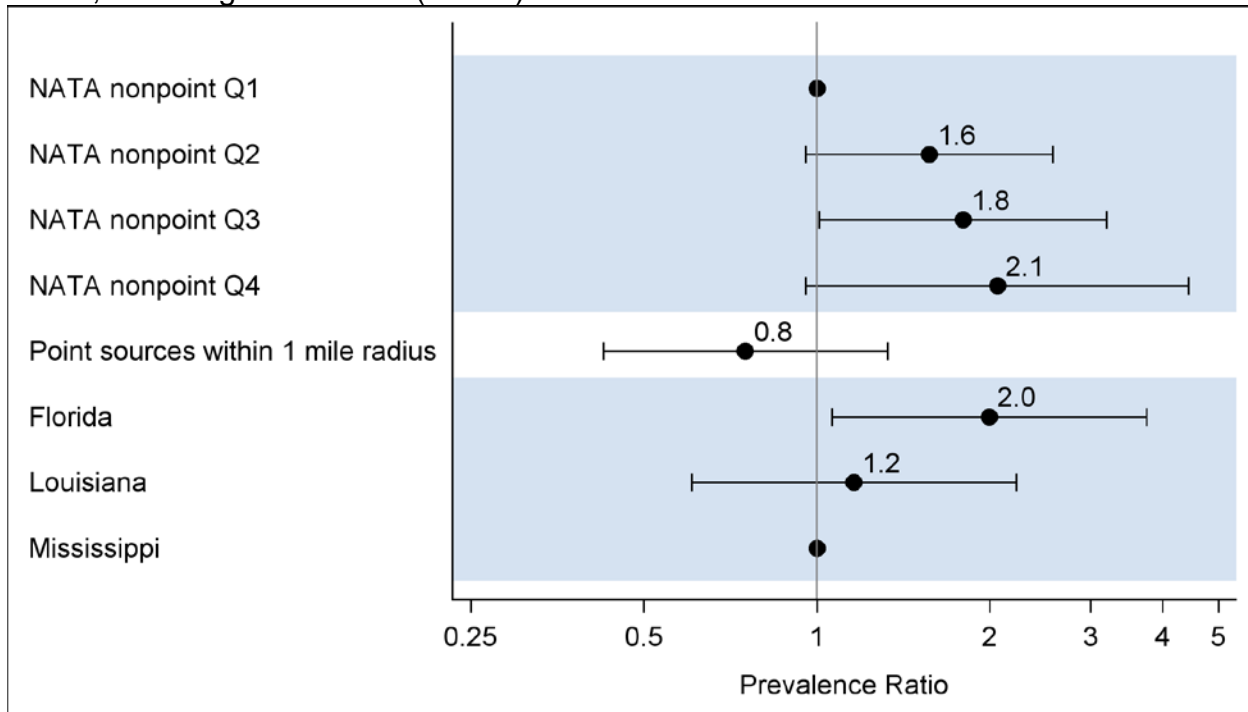
Figure 6. Predictors of blood styrene measurement above the NHANES 95th percentile (n=667).



NHANES 95<sup>th</sup> percentile is smoking-specific: smokers, 0.23 ng/mL; nonsmokers, 0.06 ng/mL.



Figure 7. Predictors of blood styrene measurement exceeding the NHANES 95th percentile among participants unexposed to tobacco smoke\* with fall/winter blood draws, not living in Alabama (n=195).



\*Unexposed to tobacco smoker: jointly defined as having blood 2,5-dimethylfuran < 0.014 ng/mL *and* reporting no active or passive smoke exposure in the 24 hours preceding blood collection.

NATA nonpoint, National Air Toxics Assessment 2011 annual average census tract ambient styrene concentration attributable to nonpoint sources.

Q1, First quartile; Q2, Second quartile; Q3; Third quartile; Q4, Fourth quartile.

Point sources, National Emissions Inventory 2011 point source emitters of styrene within 1 mile of participant's geocoded home location.

Florida, Louisiana, Mississippi, participant's state of residence.

## **CHAPTER V: STYRENE EXPOSURE AND NEUROLOGIC SYMPTOMS (SPECIFIC AIM 3)**

### **A. Introduction**

Styrene (CAS # 100-42-5, chemical formula C<sub>8</sub>H<sub>8</sub>) is an industrial hydrocarbon used in the production of plastics, fiberglass laminates, rubber, and resins found in consumer products and commercial and residential building materials. Manufactured styrene products include insulation, fiberglass boats, automotive parts, car tires, Styrofoam, and plastic drinking glasses [1]. Styrene is an established neurotoxicant at occupational levels [1, 5, 6], but has not been studied at environmental levels experienced by the general population. Epidemiologic studies to date have focused on highly exposed workers, whose average blood concentrations are 25 times higher than those of the general population [7-14].

Non-occupational exposure to styrene in the general population occurs primarily through inhalation of tobacco smoke, off-gassing of building materials, and vehicle and industrial emissions [2, 3]. Inhalation of airborne styrene accounts for over 90% of such exposures [6, 74], and is the main route of exposure resulting in health effects due to styrene toxicity [24]. Among smokers, cigarettes are considered the dominant source of styrene exposure, and smoking is the single most important individual predictor of human exposure to styrene [1, 20, 51, 59, 60, 64, 84, 85]. Styrene is released into the air from automobile exhaust, cigarette smoke, and photocopiers and printers, and industries using or manufacturing styrene. Styrene is commonly detected in urban air,

near industrial sites and landfills, and in high traffic areas, although typically at levels substantially lower than in occupational settings. Rural and suburban air generally contains lower concentrations of styrene than urban air [22].

In humans, approximately 70% of inhaled styrene is absorbed [6]. Styrene is distributed throughout the body, with the highest concentration generally found in adipose tissue [1]. Because the half-life of styrene in blood is approximately 13 hours [5], blood styrene measurements reflect recent exposure.

Though styrene's genotoxic properties have been well characterized and it is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC) [6], the Agency for Toxic Substances and Disease Registry (ATSDR) has identified the central nervous system as the primary target for styrene toxicity, with less marked effects in the peripheral nervous system [2, 99]. Like many other volatile organic compounds, styrene monomer is a central nervous system depressant with anesthesia-like properties [6, 100]. Acute solvent-induced neurotoxicity, including that caused by styrene, is characterized by symptoms of acute intoxication, commonly described as a feeling of drunkenness. Long term exposures at levels found in occupational settings have been associated with chronic adverse neurotoxic effects.

Occupational studies demonstrate styrene-induced neurotoxicity, from both acute and chronic inhaled exposure among highly-exposed workers. Symptoms include feeling "drunk" and tiredness [41], impaired vision [99, 102], vestibular dysfunction [12], headaches [103], delayed reaction time [104, 105], impaired attention and memory [9], hearing deficits [106], diminished nerve conduction velocity [9, 107-110], and abnormal EEG results [110, 111]. Similar effects have been observed at lower occupational

exposure levels, ranging from 10-30 ppm, in most [10, 99, 103-105, 114, 117-119], though not all [13, 120], studies.

The human health effects of chronic styrene exposure at typical environmental levels among the general population remain largely unknown [20]. We set out to assess the associations between two metrics of styrene exposure - estimated ambient concentrations and measured blood levels - and self-reported neurologic symptoms. Quantifying the association between environmental styrene exposure and highly sensitive, but non-specific, neurologic symptoms may lend insight into early manifestations of environmentally induced neurotoxicity due to chronic exposures at levels insufficient to cause clinically apparent toxicity.

## **B. Methods**

### **Study Design and Participants**

We used data from the Gulf Long-term Follow-up Study (GuLF STUDY), a prospective cohort of adults (ages 21 and older) who participated in oil spill response activities and others who received safety training, but were not hired, following the *Deepwater Horizon* disaster. A detailed description of this study is available elsewhere [167]. Of the 25,848 English- or Spanish-speaking GuLF STUDY participants living in the Gulf region (Alabama, Florida, Louisiana, Mississippi, and Texas) at enrollment, 24,903 reported addresses that were successfully geocoded to a 2010 U.S. Census tract. From this sample of participants with known residential locations, we excluded participants with any missing neurologic symptom information (n=304), missing demographic characteristics (n=573), and missing covariate information (n=201), leaving 23,825 eligible participants. Because autonomic and peripheral neuropathy are

known complications of diabetes [188], we further restricted the study sample to participants with no self-reported physician diagnosis of diabetes (exclude 1,825 diabetics and 38 with missing diagnosis information), resulting in a final analytic sample of 21,962.

Approximately 2-3 years after the oil spill (May 2012-July 2013), a subset of GuLF STUDY participants (N=1,055) living in the Gulf region were enrolled in the Chemical Biomonitoring Study (CBS) (Werder EJ, *in press*). CBS participants provided an extra blood sample for measuring styrene and other compounds and completed a questionnaire about usual and recent exposures.

Ultimately, 994 participants provided blood samples sufficient for quantification of styrene levels. Of those, we excluded 20 participants missing neurologic symptom information, nine with incomplete demographic characteristics, and four individuals missing other covariate information, leaving 961 eligible participants. We then excluded known diabetics (n=86 of the 1,825 diabetics from the parent study) or those missing diagnosis information (n=1), for a final analytic sample of 874.

Participants provided written consent, and the Institutional Review Board of the National Institute of Environmental Health Sciences approved this study.

### **National Air Toxics Assessment**

The United States Environmental Protection Agency (EPA) 2011 National-scale Air Toxics Assessment (NATA) [189] evaluates 180 air toxics across the United States using emissions inventories, dispersion modeling, photochemical modeling, exposure modeling, and toxicity analyses. NATA generates annual average ambient air toxic concentrations ( $\mu\text{g}/\text{m}^3$ ) for each U.S. census tract. We employed NATA styrene

estimates as indicators of typical, long-term environmental exposure by mapping each participant's geocoded home location to a corresponding 2010 U.S. census tract. Geocodes were based on self-reported home address at enrollment. The 2011 NATA annual average ambient styrene concentration corresponding to an individual's home census tract was applied as the estimate of ambient styrene exposure for each cohort member residing in the Gulf region.

### **Blood styrene measurement**

Blood collection tubes containing potassium oxalate and sodium fluoride anticoagulant were used to collect 10 mL of blood for styrene measurement. Tubes and stoppers were pre-treated by the Centers for Disease Control and Prevention (CDC) laboratory to remove VOCs residues to minimize pre-collection contamination [175, 176]. Samples were stored in a 4°C refrigerator prior to being shipped overnight on cold packs in biweekly batches to the Division of Laboratory Sciences, National Center for Environmental Health, CDC in Atlanta, Georgia, for analysis of VOCs. Analysis of styrene followed standard CDC procedures, using equilibrium headspace solid-phase micro-extraction with benchtop gas chromatography/mass spectrometry [168, 170].

### **Neurologic symptoms**

Health information, including questions about neurologic symptoms, was collected at enrollment via Computer Assisted Telephone Interview (CATI) during the baseline interview. All participants were asked to report how often they experienced dizziness, lightheadedness, vision impairment, numbness, tingling, stumbling while

walking, headaches, and fatigue during the preceding 30 days. Three additional *episodic* outcomes, *i.e.*, seizures, vomiting, and insomnia, were added to the questionnaire after data collection was underway. These latter outcomes were not combined with other symptoms for evaluation of “any” neurologic symptom or symptom clusters because their enumeration in the study population is incomplete (*i.e.*, those people who completed the enrollment interview prior to the inclusion of questions about seizures, vomiting, and insomnia did not have the opportunity to report them). We did, however, analyze these additional symptoms individually among the subgroup of participants who had the opportunity to report them.

Frequency of most symptoms was reported as: *all of the time, most of the time, sometimes, rarely, or never*. Symptoms were classified as a binary indicator of the ‘presence’ (all or most of the time) or ‘absence’ (sometimes, rarely, or never) of occurrence. Seizures, vomiting and insomnia were reported as: *every day, several times a week, once a week, rarely, or never*. We applied a lower threshold for the reporting of episodic symptoms because the relative frequency of their occurrence is expected to be lower, as compared with potentially ongoing symptoms. Accordingly, the presence of vomiting included responses of once a week or more, and the presence of seizures included reporting a seizure at any point during the 30 days (rarely, once a week, several times a week, every day).

## **Statistical analysis**

We used multivariate log-binomial regression to estimate prevalence ratios and corresponding 95% confidence intervals (PR, 95% CI) for the cross-sectional

associations between modeled and measured styrene exposure and neurologic symptom prevalence. We evaluated associations between (i) ambient styrene exposure ( $\mu\text{g}/\text{m}^3$ ) and neurologic symptoms among all eligible study participants residing in the Gulf region (N=21,962), and (ii) blood styrene concentration (ng/mL) and neurologic symptoms among CBS participants (n=874). We also examined the distributions of NATA ambient concentrations and log-transformed blood styrene levels to determine relevant rank-based exposure metrics.

We analyzed symptom clusters as primary outcomes. Based on results of a latent class analysis of symptom correlations among all reported symptoms (data not shown), we identified two neurologic clusters (*i.e.*, CNS and PNS). The CNS cluster included dizziness, headache, nausea, sweating, and palpitations. The PNS cluster included tingling and numbness in the extremities, blurred vision, and stumbling while walking. For the main analyses, we separately examined associations between styrene exposure and the presence of any neurologic symptom, any CNS symptom, more than one CNS symptom, any PNS symptom, and more than one PNS symptom. We included sweating and palpitations in our identification of CNS symptoms, based on results of the latent class analysis. We did not, however analyze these symptoms as individual neurologic outcomes in any other analyses because of their non-specific nature and lack of precedent in neurotoxicity literature. We further completed sensitivity analyses excluding sweating and palpitations from the CNS symptom cluster. In secondary analyses, we examined associations between styrene exposure and each individual neurological symptom in a separate model.



All models were adjusted for sex (male, female), age (<30, 30-45, >45), season (spring, summer, fall, winter), race (white, black, other), employment status (currently working, not working), alcohol drinking (current, former/never), and self-reported smoking (current, former/never). Models assessing associations with blood styrene were additionally adjusted for the duration of time (days) between enrollment (symptom ascertainment) and the date of blood styrene collection. The median lag between enrollment and blood styrene collection was 100 days. For statistical analyses, we used all measured blood styrene values, including the actual values below the LOD [180]. Covariates were selected based on directed acyclic graph analysis [190] of the theoretical relationship between styrene exposure and neurologic symptoms. Covariate information was obtained during the enrollment interview.

We conducted several sensitivity analyses, examining the impact of depression, co-exposures, socioeconomic status, and amount of overall symptom reporting (*i.e.*, number of symptoms reported) on observed associations. Depression was defined based on self-reported physician diagnosis at enrollment, and we conducted stratified analyses comparing associations between participants who did and did not report depression. For co-exposure analyses, we additionally adjusted for benzene and toluene, in separate models. Ambient benzene and toluene estimates were abstracted from NATA 2011, and blood benzene and toluene levels were measured on the same panel as styrene. In ambient analyses, we additionally adjusted for ambient particulate matter (PM 2.5). Annual census tract estimates of PM 2.5 were obtained from the EPA fused Downscaler Model 2011 output [191]. To address socioeconomic status, we adjusted for income, education, and self-reported concerns over affording housing and

food. Participants were asked about frequency of 18 symptoms in addition to those included here as indicative of potential neurological effects. We tried several approaches to account for symptom over-reporting, including making exclusions for different thresholds of number of symptoms reported, adjusting for total number of symptoms reported, excluding participants who reported excessive recent hair loss, which is not believed to be related to any of the exposures under investigation, and restricting analyses to participants reporting only neurologic symptoms. We also completed analyses specifying different exposure contrasts for both ambient and blood styrene exposure, as well as evaluating the impact of adjusting for diabetes instead of excluding participants reporting a diabetes diagnosis. We also repeated all main analyses for clusters and individual symptoms restricting to the subgroup of participants who had the opportunity to report on the three symptoms (seizures, vomiting, and insomnia) that were added later.

All statistical analyses were conducted in SAS 9.4 (Cary, NC, USA).

### **C. Results**

This cohort is predominantly male (80.6%), younger than 45 (58.8%), and overweight (40.4%) or obese (31.5%) (Table 6). Approximately one-third of participants are current smokers, and 75.6% completed at least one day of oil spill response or cleanup work. Participants who provided blood specimens for CBS were less likely to be employed (52.9% vs. 62.7%) and more likely to have worked on oil spill response (84.9% vs. 75.6%), compared to the full cohort of Gulf State residents. The CBS also has a higher proportion of nonwhite participants than the overall sample (49.8% compared to 37.6%), and fewer college graduates (12.0% compared to 18.5%). Among

the population eligible for the ambient styrene analyses, as well as the CBS, the distribution of demographic characteristics is similar between participants who were excluded due to incomplete covariate information and participants who were included in the respective modeled samples.

Based on the distribution of ambient styrene concentrations among Gulf region participants (Figure 8), we modeled neurologic symptoms in relation to quartiles of ambient exposure. The distribution features a prominent right skew, with the top quartile ranging from 0.03 to 1.70  $\mu\text{g}/\text{m}^3$  styrene. The referent exposure group (first quartile) experienced ambient styrene levels up to 0.01  $\mu\text{g}/\text{m}^3$  styrene. When comparing airborne styrene concentrations in the Gulf region to the entire U.S., the exposure distributions are very similar with some slight variability in the top 5% of census tracts (data not shown). The distribution of blood styrene concentration has a less pronounced right skew (Figure 1) and the sample size is relatively small. We elected to dichotomize blood concentrations at the median value, 0.067 ng/mL styrene.

Increasing ambient styrene concentration was modestly associated with the presence of any neurologic symptom, though the association was only significant for the fourth quartile of exposure (PR, 1.07; 95% CI: 1.02, 1.12) (Figure 9). For any CNS symptom, multiple CNS symptoms, any PNS symptom, and multiple PNS symptoms, the top quartile of exposure was positively and significantly associated with reporting neurologic symptoms. The strongest association we observed among the cluster analyses was a 20% increase in prevalence of multiple CNS symptoms among those in the top quartile of ambient styrene concentration (PR, 1.20; 95% CI: 1.09, 1.32). We observed significant linear trends ( $p$ -value < 0.05) between styrene exposure quartiles

and each of the symptom clusters, though trends were most apparent for any CNS symptom, multiple CNS symptoms, and multiple PNS symptoms (Table 7).

In analyses of blood styrene above the median concentration and neurologic symptom clusters, we observed small to modest, nonsignificant positive associations for multiple CNS, any PNS, and multiple PNS symptoms (Figure 10, Table 8). The strongest association was a 40% increase in prevalence of multiple PNS symptoms, which was of borderline significance (PR, 1.40; 95% CI: 1.00, 1.97).

The prevalence of individual neurologic symptoms ranged from 1.8% for seizures to 16.1% for fatigue, with 30.8% of participants reporting at least one of the original neurologic symptoms (Table 7). Among those participants who were also asked about the additional symptoms added later (n=16,536), the prevalence of any original neurologic symptom (excluding vomiting, seizure, and insomnia) was 33.0% (and 38.0% including vomiting, seizure, and insomnia). The highest quartile of ambient exposure was positively and significantly associated with each individual neurologic symptom. Many of the associations with individual symptoms demonstrated an increasing exposure-response relationship across quartiles of exposure, and linear trends were significant ( $p < 0.05$ ) for all symptoms except fatigue and nausea (Figure 11).

Among CBS participants, seizure was the least common symptom (2.8%) and fatigue was the most prevalent (24.7%). Although associations between blood styrene and symptom clusters were generally modest, we did observe noteworthy, albeit nonsignificant, positive associations for the individual symptoms of dizziness (PR, 1.36; 95% CI: 0.91, 2.04) and nausea (PR, 1.53; 95% CI: 0.93, 2.53), and a significant association for stumbling (PR, 2.14; 95% CI: 1.03, 4.44) (Table 8).

Our findings were robust to exclusions and adjustments made in sensitivity analyses. Neither excluding participants who reported a total number of symptoms exceeding a range of thresholds (e.g. the 95<sup>th</sup> percentile, or 12 symptoms), nor adjusting for total number of symptoms reported, had an appreciable impact on observed associations between ambient styrene and neurologic outcomes. Restricting the duration of time between enrollment and blood draw to below the median (100 days) attenuated some associations between blood styrene and CNS symptoms slightly, but did not meaningfully change associations. Results were generally similar in analyses using other exposure contrasts for both airborne and blood styrene exposures (e.g. tertiles, quintiles, 90<sup>th</sup> percentile, etc.), although risk estimates became more unstable as exposure groups became smaller. Excluding sweating and palpitations from the CNS symptom cluster did not meaningfully change results, though most associations were slightly stronger using this reduced CNS symptom cluster.

Correlation coefficients between ambient concentrations of styrene and each of benzene, toluene, and PM 2.5 were 0.69, 0.76, and 0.43, respectively. Associations between styrene and neurologic symptom clusters were stronger in models adjusted for ambient benzene or toluene (Figure 12). Adjusting for PM 2.5 had little effect, yielding estimates that were virtually identical to our primary results. For multiple CNS or PNS symptoms, adjustment for co-exposures left associations essentially unchanged (data not shown).

To evaluate the effect of depression on symptom reporting, we estimated associations separately for participants who reported a diagnosis of depression (n=3,069, 14%) and those who did not (n=18,805, 86%). Associations among

participants without depression, representing the majority of the sample, were similar to overall results (Figure 13).

When we repeated analyses for clusters, as well as individual symptoms, among the subgroup of participants who completed the interview after questions about seizures, insomnia, and vomiting were added, results were virtually identical to originally reported associations. Indeed, because the order in which participants were enrolled in the study was largely random, we have no reason to suspect that individuals who responded before the additional symptoms were included would be meaningfully different from those who responded after their inclusion.

#### **D. Discussion**

To our knowledge, this study was the first to assess subclinical neurotoxicity of styrene exposure at environmental levels relevant to the general population. We observed consistent, positive relationships between increasing ambient styrene exposure and neurologic symptoms. Associations were consistently statistically significant among the highest exposure groups and a monotonic exposure-response was evident for many outcomes. Although associations between blood styrene and neurologic symptom clusters were generally not apparent, we did observe suggestive effects for the peripheral nervous system cluster and some individual symptoms (dizziness, nausea, and stumbling).

Among the analyses of ambient exposure and symptom clusters, we observed slightly stronger associations with an unambiguous dose response and significant linear trends for the central nervous system cluster. These findings are supported by ASTDR's

report identifying the central nervous system as the primary target of styrene toxicity [1], as well as increased mortality from diseases of the central nervous system, especially epilepsy, associated with styrene exposure in a cohort study of reinforced-plastics industry workers [121].

When examining individual neurologic symptoms, we observed the strongest associations for ambient styrene with reported seizures, blurred vision, stumbling, dizziness, and insomnia. Symptoms with the strongest evidence for styrene-induced neurotoxicity at occupational levels include blurred or distorted vision [102, 149, 192-195], dizziness or lightheadedness [13, 103, 117], headaches [41, 103, 119], and fatigue [41, 103, 117, 119]. Less consistent associations have been reported for vestibular impairment [119], tingling and numbness [11, 103, 105], seizures [105, 196], and insomnia [197]. Despite much lower ambient exposure levels in our study than those present in occupational settings, our results are generally consistent with these findings.

Blood styrene levels among CBS participants were two to three times higher than those measured in the National Health and Nutrition Examination Survey, for smokers and nonsmokers alike. We observed suggestive associations of blood styrene concentration in relation to dizziness, nausea, stumbling, and having multiple peripheral nervous system symptoms. These effect estimates were larger in magnitude, but some lacked precision due to the sample size and frequency of symptom reporting.

Having two or more peripheral nervous system symptoms was associated with both ambient and blood concentrations of styrene. Dizziness, nausea, and stumbling also demonstrated some consistency between elevated ambient and biomarker

exposures. Differences in associations between exposure types may be attributable to the relevant temporal window each exposure type captures. Measured blood styrene generally reflects the previous 24 hours, whereas NATA estimates are annual averages thought to reflect longer term exposure. Blood samples were drawn, on average 100 days after symptom information was collected. Thus, exposure misclassification is especially likely to have limited our ability to detect significant associations.

Our study is the first to investigate whether neurologic effects observed among highly exposed occupational populations are also observed among individuals with styrene exposure more typical of the general population. Recent environmental studies evaluating effects of simultaneous exposure to multiple hazardous air pollutants (HAPs) have documented associations between environmental styrene and other neurologic outcomes. When evaluating modeled ambient exposure estimates for a variety of HAPs, styrene was associated with increased risk of autism spectrum disorder [122, 123] and amyotrophic lateral sclerosis [124]. These results implicate low-level, chronic styrene exposure as a possible public health problem. However, a cross-sectional analysis of blood VOCs and neurobehavioral testing found a general lack of significant adverse effects, with the exception that a mixture of BTEX and styrene was modestly associated with slower reaction time [125].

Studies in humans and experimental *in vitro* and *in vivo* animal models have attempted to determine the mode of action for styrene neurotoxicity, with a dopaminergic mechanism gaining traction [71], but explanations remain speculative. Several studies suggest that styrene exposure alters dopamine metabolism, marked by decreased dopamine levels and increased dopamine receptors in rodents and humans



[126-128]. The styrene metabolites phenylglyoxylic acid and mandelic acid were shown to deplete dopamine in neurologic tissues [129]. This mechanism is corroborated in blood samples of styrene-exposed plastics workers for whom prolactin levels are elevated, as prolactin release is chronically inhibited by dopamine [198]. Consistent with disturbance of the dopaminergic functions of the brain, styrene exposure potentiates a dose-dependent decrease in brain dopamine in male rats [128]. Styrene also caused cell loss and dopamine depletion in retinas isolated from female rats [132], which supports the established association between occupational styrene exposure and impaired vision [133].

Our study has several strengths, including a large sample size, a well-characterized, diverse, understudied population, multiple sensitive measures of neurotoxicity, complementary metrics of exposure, and findings that were robust to multiple sensitivity analyses.

Neurologic symptoms can reveal subtle impairments in neurologic function before the occurrence of clinically apparent disease. While less severe, these symptoms may be more sensitive to lower exposure levels, longer lasting, more prevalent in the general population, and possibly persist after exposure recedes. Self-reported symptoms, despite being a subjective endpoint, can provide highly sensitive measures of neurologic and toxicant-associated neurotoxicity [199]. Thus, symptom assessment is an appropriate measure to capture the potentially subtle, widespread neurotoxic effects of the observed environmental exposure levels.

We assessed two metrics of styrene exposure, one reflecting long-term environmental exposure and one reflecting short-term internal burden. Modeling

exposure this way helps address the trade-offs between possible confounding bias for blood styrene, and potential measurement error of NATA ambient styrene estimates [200]. Associations were not entirely consistent between blood and ambient styrene measures, which may be due to limitations of both biomarkers and proxy exposure measures, or the temporal windows they reflect. NATA estimates have been successfully used as measures of human air pollution exposure in epidemiologic studies of cancer [201-203], asthma [204], birth defects [205], autism spectrum disorder [122, 123, 206], and neurodegenerative diseases [124]. We used NATA styrene estimates as indicators of typical, long-term environmental exposure, and blood styrene measurements to capture internal burden resulting from recent exposures. Blood styrene is a validated biomarker specific to styrene exposure [74] and it has been used extensively in occupational research [6], as well as in general population monitoring [1].

Limitations of our study include the use of an annual average ambient concentration, single blood draw for styrene measurement, and lack of detailed occupational exposure information. Due to the cross-sectional study design, we cannot confirm temporality between styrene exposure and the onset of neurologic symptoms. It is, however, unlikely that the symptoms assessed would lead to the observed ambient or blood styrene concentrations we observed. More relevant to the cross-sectional design limitation is our inability to distinguish between transient effects due to acute exposures and persistent neurologic effects of long-term styrene exposure.

Routine monitoring of ambient styrene in the U.S. does not provide sufficient temporal or spatial coverage to support exposure interpolation methods [143, 145, 155]. NATA remains the only spatially-referenced exposure data source with sufficient

geographic coverage for the Gulf state region. Although the assumptions inherent to an annual average estimate of air pollution potentially limit interpretation for acute exposure scenarios, NATA data are a valid estimation of usual exposure levels. If styrene-associated neurologic effects are the result of long-term exposure, this type of exposure metric may be better suited to capturing the associations of interest. Long-term ambient styrene trends indicate that year-to-year regional variation in concentration is not substantial, suggesting that lifetime average estimates are appropriate assessments of exposure [20]. Stable blood styrene levels in independent U.S. population samples, measured cross-sectionally, over a 20-year period corroborate these findings [60, 66, 74].

Although we obtained only a single blood measurement from each individual, the sample size is large for such a biomarker-based study. At occupational exposure levels, biomarkers consistently predict exposure classification according to job title and occupational styrene-related activities [7, 10, 38-47]. In general, these biomarkers correlate well with indoor air styrene levels, but the relationship does not necessarily persist for outdoor air styrene concentrations. Indeed, we observed limited concordance between measured blood styrene and NATA estimated concentrations (Werder EJ, *submitted*). In occupational studies, the correlation between styrene in blood and indoor air ranges from 0.62 to 0.94 [14, 46, 72]. In a study of low occupational styrene levels measured in air, blood, and urine, the best correlations with ambient levels were observed for blood styrene [45].

Lacking detailed information on styrene-specific occupational exposure opportunities, we assessed reported industry, occupation, and activity information for

participant's recent and longest-held jobs. We searched for any potentially styrene-related work experience, with the intention of adjusting for work or excluding these individuals from analyses. Ultimately, occupational styrene exposure opportunities were so rare in our study population (0.1%), that we concluded that occupational exposures were not appreciably influencing results.

Our results provide strong evidence for an association between ambient styrene exposure and neurologic symptoms, suggesting that styrene may be neurotoxic at exposure levels relevant to the general population. These findings were consistent and robust across a wide range of sensitivity analyses. The relationship between blood styrene exposure and neurologic symptoms was equivocal, but suggestive of an association for certain endpoints. Due to the cross-sectional nature of our study, the temporality of exposure and outcome is uncertain. While short-term acute intoxication is reversible, ceasing when styrene is cleared from the body, more concerning for overall health are the potential chronic, subtle but demonstrative, and irreversible effects that persist after styrene is cleared from the body [101]. If, however, environmental styrene exposure is geographically pervasive and temporally persistent, then transient effects will emulate chronic effects. Timing of environmental styrene exposure and duration of neurotoxic effects may be an important area of future public health research.

## **E. Conclusions**

Our results provide compelling evidence for an association between ambient styrene exposure and neurologic symptoms, suggesting that styrene's neurotoxic properties may persist at exposure levels relevant to the general population. These findings were consistent and robust to exhaustive sensitivity analyses. The relationship

between blood styrene exposure and neurologic symptoms was equivocal, but suggestive of an association for certain endpoints. Due to the cross-sectional nature of our study, we can't evaluate the relevant timing of exposure and outcome. While short-term acute intoxication is reversible, ceasing when styrene is cleared from the body, more concerning for overall health are the potential chronic, subtle but demonstrative, and irreversible effects that persist after styrene is cleared from the body [101]. Timing of environmental styrene exposure and duration of neurotoxic effects may be an important area of future public health research.

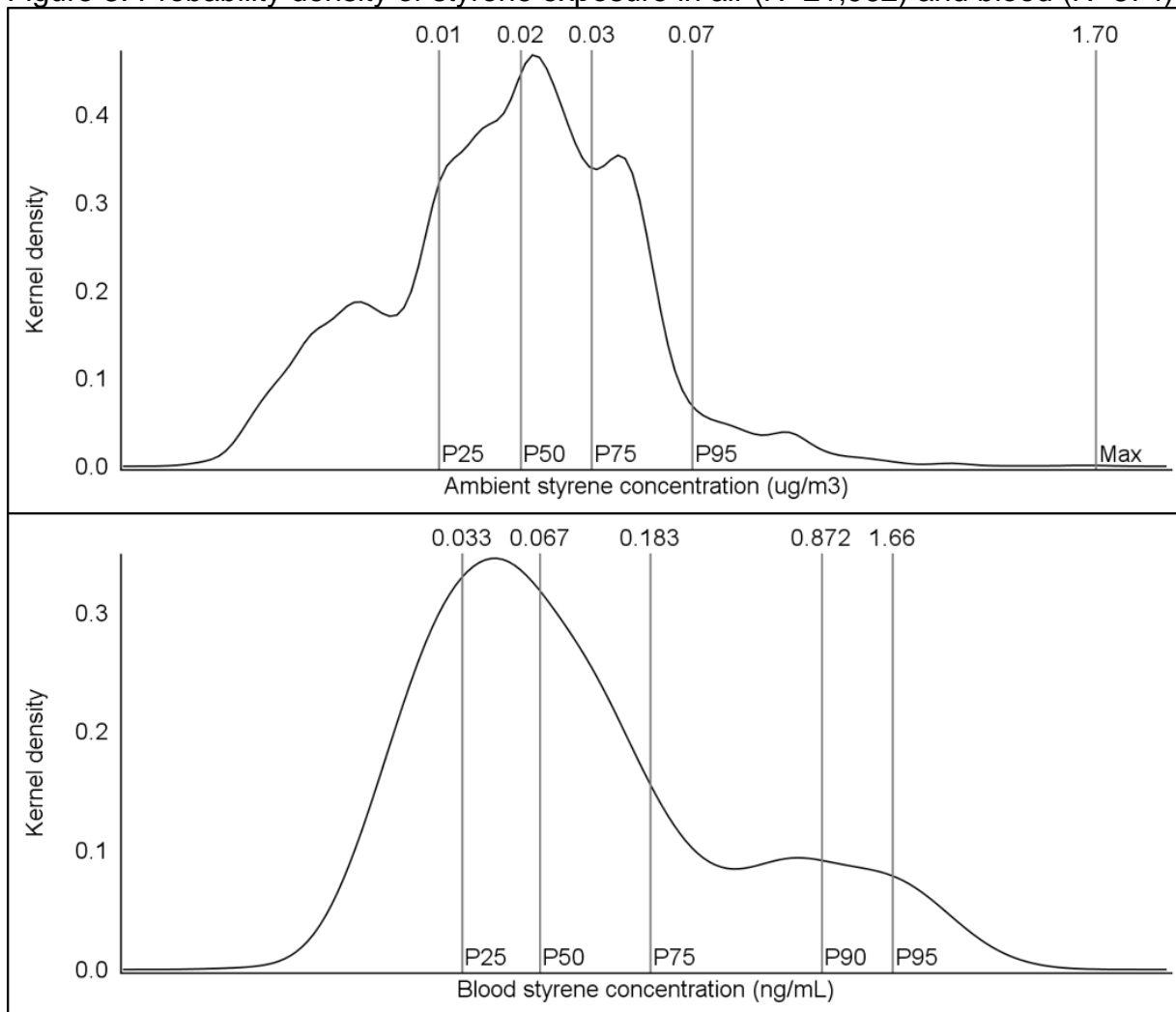
## F. Tables and Figures

Table 6. Demographic characteristics of participants living in the Gulf States (N=21,962) and those participating in the Chemical Biomonitoring Study (CBS, N=874).

Characteristic		Gulf States (n=21,962)		CBS (n=874)	
		N	%	N	%
Sex	Female	4,259	19.4	220	25.2
	Male	17,703	80.6	654	74.8
Age, years	< 30	4,685	21.3	194	22.2
	30 - 45	8,241	37.5	343	39.2
	< 45	9,036	41.1	337	38.6
Season of enrollment	Spring	6,104	27.8	174	19.9
	Summer	5,329	24.3	198	22.7
	Fall	4,968	22.6	185	21.2
	Winter	5,561	25.3	317	36.3
Race	White	13,723	62.5	439	50.2
	Black	6,051	27.6	368	42.1
	Other	2,188	10.0	67	7.7
Work status	Employed	13,780	62.7	462	52.9
	Unemployed	8,182	37.3	412	47.1
Current drinker	Yes	16,441	74.9	598	68.4
	No	5,521	25.1	276	31.6
Current smoker	Yes	7,514	34.2	261	29.9
	No	14,448	65.8	613	70.1
Oil spill response work <sup>1</sup>	≥ 1 day	16,599	75.6	742	84.9
	None	5,363	24.4	132	15.1
Education	< High school	4,072	18.5	179	20.5
	High school graduate	7,285	33.2	340	38.9
	Some college	6,554	29.8	250	28.6
	≥ College graduate	4,051	18.5	105	12.0
Body Mass Index, kg/m <sup>2</sup>	≤ Normal (< 25)	6,173	28.1	249	28.5
	Overweight (25 - < 30)	8,877	40.4	333	38.1
	Obese (≥ 30)	6,912	31.5	292	33.4

<sup>1</sup>Participants reported working on Deepwater Horizon oil spill cleanup for at least one day in 2010-2011.

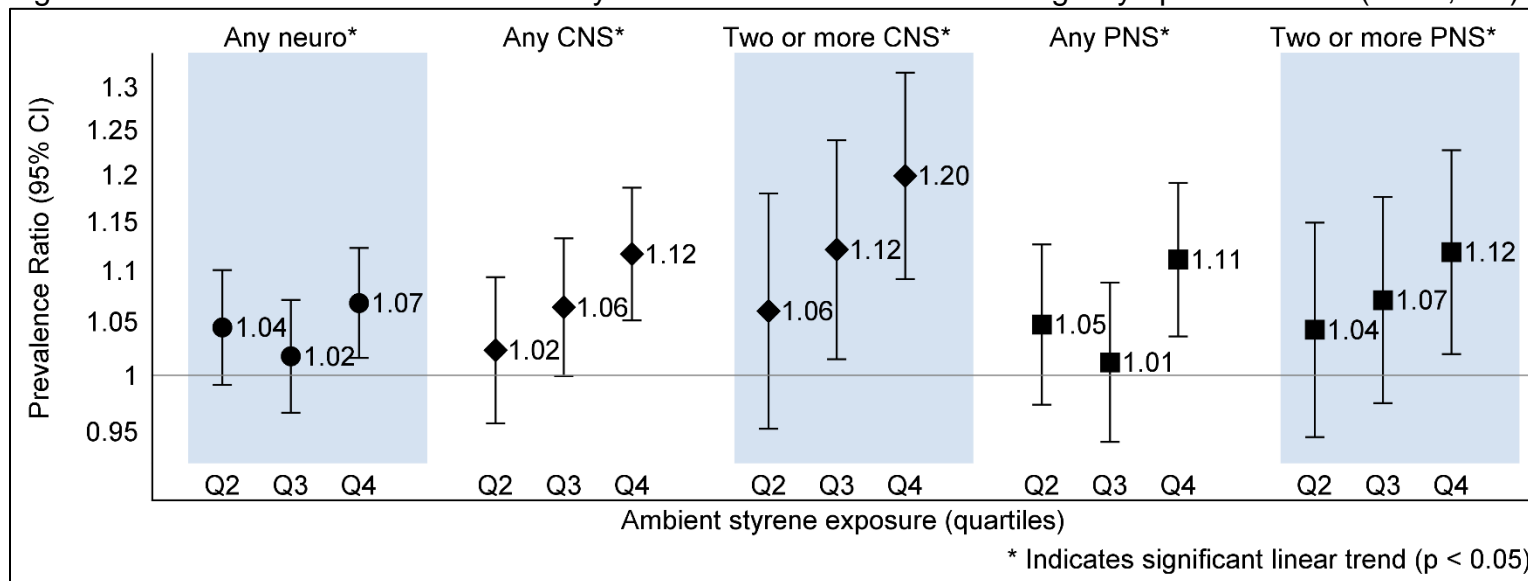
Figure 8. Probability density of styrene exposure in air (N=21,962) and blood (N=874).



Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average concentrations ( $\mu\text{g}/\text{m}^3$ ) at the census tract level. Blood styrene exposure concentrations (ng/mL) are measured from a single blood draw obtained in the participant's home.

Values at the top of reference lines indicate exposure concentrations; labels at the bottom of reference lines indicate locations in the exposure distribution: P25, 25<sup>th</sup> percentile; P50, 50<sup>th</sup> percentile; P75, 75<sup>th</sup> percentile; P90, 90<sup>th</sup> percentile; P95, 95<sup>th</sup> percentile; Max, maximum value.

Figure 9. Association between ambient styrene concentration and neurologic symptom clusters (N=21,962).



Ambient styrene exposure quartiles are based on National Air Toxics Assessment (NATA) 2011 estimates of annual average concentrations at the census tract level; referent exposure group is the lowest quartile (not shown on figure).

Q2, second quartile of exposure; Q3, third quartile; Q4 fourth (highest) quartile.

Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, and smoking status at enrollment.

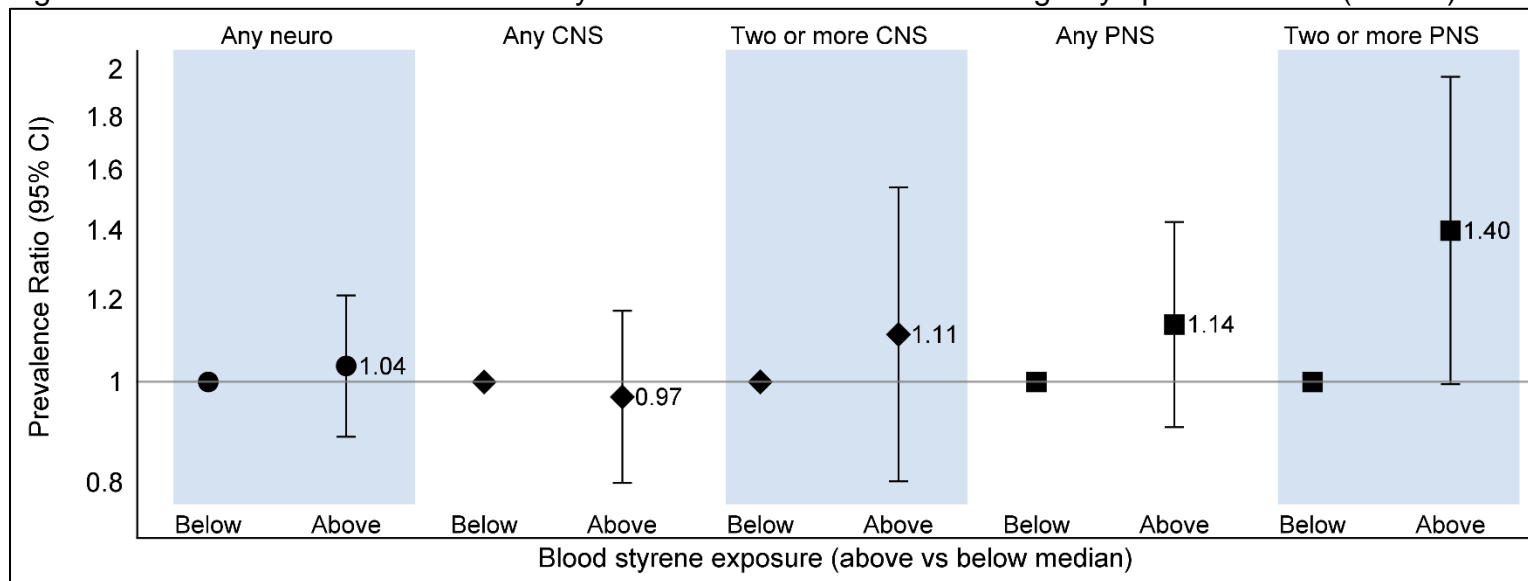
Central Nervous System (CNS) symptoms include: dizziness, headache, nausea, sweating, and palpitations.

Peripheral Nervous System (PNS) symptoms include: tingling/numbness, blurred vision, and stumbling.

Asterisks (\*) indicate p-value for linear trend < 0.05.



Figure 10. Association between blood styrene concentration and neurologic symptom clusters (N=874).



Blood styrene exposure concentrations are measured from a single blood draw obtained in the participant's home, and exposure is classified as a measurement above or below the median value (0.067 ng/mL).

Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, smoking status at enrollment, and duration (days) between enrollment and blood draw.

Central Nervous System (CNS) symptoms include: dizziness, headache, nausea, sweating, and palpitations.

Peripheral Nervous System (PNS) symptoms include: tingling/numbness, blurred vision, and stumbling.

Table 7. Association between ambient styrene concentration and individual neurologic symptoms (N=21,962).

Outcome	Prevalence, N (%)	Exposure	PR (95% CI)	p-trend
Any neurologic symptom	6,759 (30.8)	Q2	1.04 (0.99,1.10)	0.03
		Q3	1.02 (0.97,1.07)	
		Q4	1.07 (1.02,1.12)	
Any CNS symptom <sup>1</sup>	4,960 (22.6)	Q2	1.02 (0.96,1.09)	0.0001
		Q3	1.06 (1.00,1.13)	
		Q4	1.12 (1.05,1.19)	
≥ 2 CNS symptoms	2,037 (9.3)	Q2	1.06 (0.95,1.18)	0.0001
		Q3	1.12 (1.02,1.24)	
		Q4	1.20 (1.09,1.32)	
Dizziness	1,468 (6.7)	Q2	0.98 (0.86,1.12)	0.00001
		Q3	1.09 (0.97,1.24)	
		Q4	1.26 (1.12,1.40)	
Nausea	1,046 (4.8)	Q2	1.01 (0.87,1.17)	0.10
		Q3	0.91 (0.79,1.06)	
		Q4	1.16 (1.02,1.32)	
Headache	3,121 (14.2)	Q2	1.06 (0.97,1.15)	0.001
		Q3	1.10 (1.02,1.19)	
		Q4	1.13 (1.05,1.22)	
Any PNS symptom <sup>2</sup>	4,014 (18.3)	Q2	1.05 (0.97,1.13)	0.01
		Q3	1.01 (0.94,1.09)	
		Q4	1.11 (1.04,1.19)	
≥ 2 PNS symptoms	2,429 (11.1)	Q2	1.04 (0.95,1.15)	0.01
		Q3	1.07 (0.98,1.18)	
		Q4	1.12 (1.02,1.23)	
Tingling/Numbness	3,146 (14.3)	Q2	1.05 (0.96,1.14)	0.01
		Q3	1.04 (0.96,1.14)	
		Q4	1.12 (1.03,1.21)	
Stumble	739 (3.4)	Q2	1.12 (0.93,1.34)	0.008
		Q3	1.11 (0.93,1.32)	
		Q4	1.27 (1.07,1.50)	
Blurred vision	1,656 (7.5)	Q2	1.10 (0.97,1.25)	<.0001
		Q3	1.12 (1.00,1.27)	
		Q4	1.36 (1.21,1.52)	
Fatigue	3,529 (16.1)	Q2	1.08 (0.99,1.16)	0.08
		Q3	1.02 (0.94,1.10)	
		Q4	1.10 (1.02,1.18)	
Insomnia <sup>3</sup>	1,552 (13.8)	Q2	1.18 (1.04,1.34)	0.002
		Q3	1.18 (1.04,1.34)	
		Q4	1.23 (1.08,1.39)	

Outcome	Prevalence, N (%)	Exposure	PR (95% CI)	p-trend
Vomit <sup>3</sup>	1,363 (9.3)	Q2	1.13 (0.99,1.30)	0.01
		Q3	1.15 (1.01,1.31)	
		Q4	1.17 (1.03,1.33)	
Seizure <sup>3</sup>	263 (1.8)	Q2	1.12 (0.80,1.55)	0.005
		Q3	1.17 (0.85,1.60)	
		Q4	1.51 (1.12,2.02)	

Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, and smoking status at enrollment.

Prevalence indicates total prevalence in the study sample (all exposure levels).

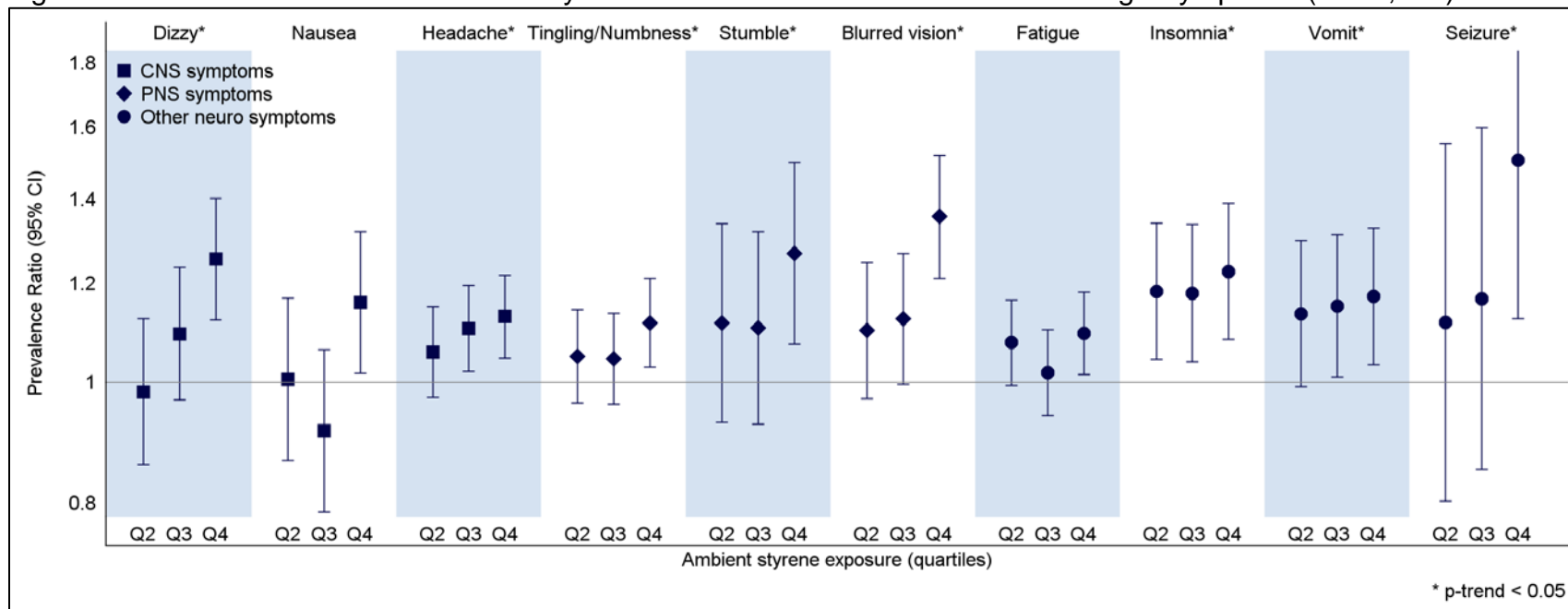
Ambient styrene exposure quartiles are based on National Air Toxics Assessment (NATA) 2011 estimates of annual average concentrations at the census tract level; referent exposure group is the lowest quartile (not shown in table). Q2, second quartile of exposure; Q3, third quartile; Q4 fourth (highest) quartile.

<sup>1</sup>Central Nervous System (CNS) symptoms: dizziness, headache, nausea, sweating, palpitations.

<sup>2</sup>Peripheral Nervous System (PNS) symptoms: tingling/numbness, blurred vision, and stumbling.

<sup>3</sup>Sample sizes vary for insomnia (n=11,236), vomiting (n=14,591), and seizure (n=14,591) because they were added to the interview after data collection was underway, and they are therefore ineligible for inclusion in symptom groupings (Any neurologic/CNS/PNS).

Figure 11. Association between ambient styrene concentration and individual neurologic symptoms (N=21,962).



Ambient styrene exposure quartiles are based on National Air Toxics Assessment (NATA) 2011 estimates of annual average concentrations at the census tract level; referent exposure group is the lowest quartile (not shown on figure). Q2, second quartile of exposure; Q3, third quartile; Q4 fourth (highest) quartile.

Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, and smoking status at enrollment.

Central Nervous System (CNS) symptoms include: dizziness, headache, nausea, sweating, and palpitations.

Peripheral Nervous System (PNS) symptoms include: tingling/numbness, blurred vision, and stumbling.

Table 8. Association between blood styrene concentration and individual neurologic symptoms (N=874).

Outcome	Prevalence, N (%)	PR (95% CI)
Any neurologic symptom	434 (49.7)	1.04 (0.89,1.21)
Any CNS symptom <sup>1</sup>	274 (31.4)	0.97 (0.80,1.17)
≥ 2 CNS symptoms	128 (14.7)	1.11 (0.80,1.54)
Dizziness	90 (10.3)	1.36 (0.91,2.04)
Nausea	80 (9.2)	1.53 (0.93,2.53)
Headache	186 (21.3)	1.01 (0.78,1.31)
Any PNS symptom <sup>2</sup>	240 (27.5)	1.14 (0.90,1.43)
≥ 2 PNS symptoms	152 (17.4)	1.40 (1.00,1.97)
Tingling/numbness	190 (21.7)	1.07 (0.81,1.40)
Stumbling	45 (5.2)	2.14 (1.03,4.44)
Blurred vision	105 (12.0)	0.99 (0.68,1.42)
Fatigue	216 (24.7)	0.94 (0.73,1.20)
Insomnia <sup>3</sup>	130 (16.7)	1.11 (0.79,1.54)
Vomiting <sup>3</sup>	112 (13.1)	1.03 (0.73,1.45)
Seizure <sup>3,4</sup>	24 (2.8)	—

Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, smoking status at enrollment, and duration (days) between enrollment and blood draw.

Blood styrene exposure is classified as above the median concentration (0.067 ng/mL) compared to all measurements below the median concentration.

<sup>1</sup>Central Nervous System (CNS) symptoms include: dizziness, headache, nausea, sweating, and palpitations.

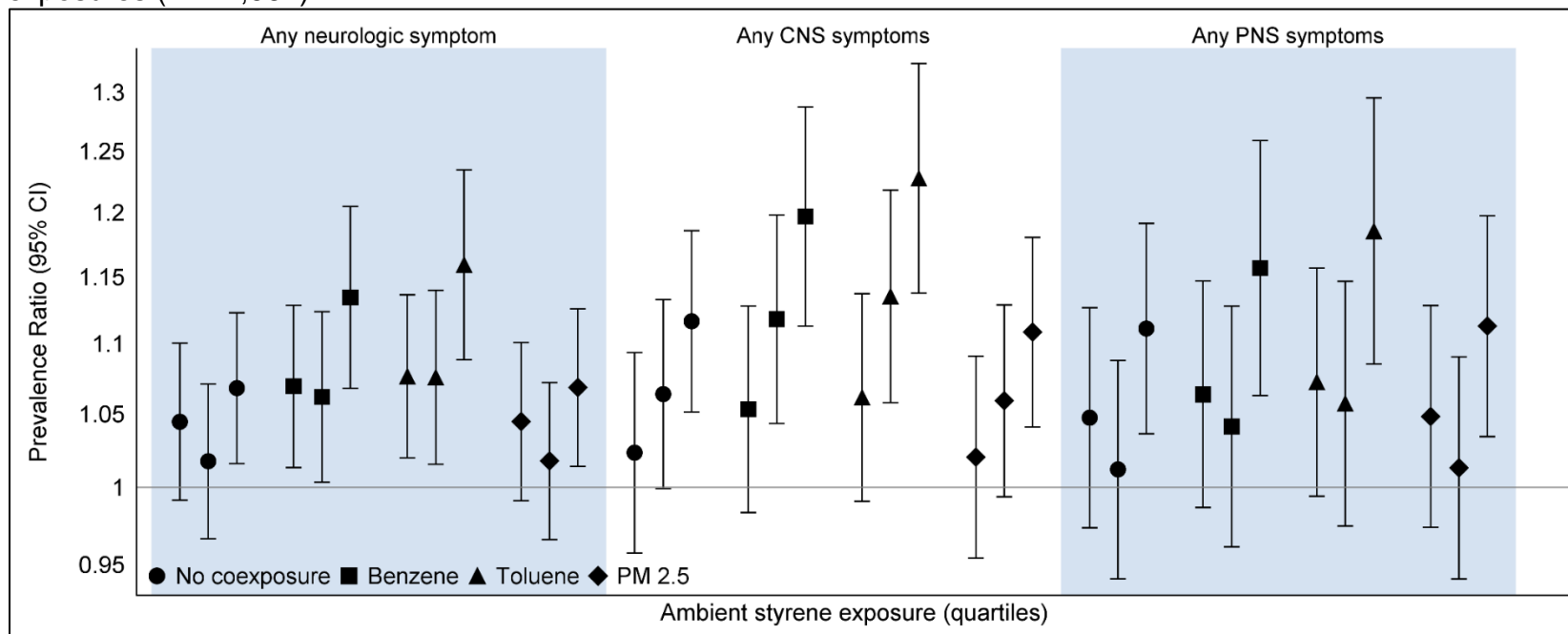
<sup>2</sup>Peripheral Nervous System (PNS) symptoms include: tingling/numbness, blurred vision, and stumbling.

Prevalence indicates total prevalence in the study sample (both exposed and unexposed).

<sup>3</sup>Sample sizes vary for insomnia (n=780), vomiting (n=857), and seizure (n=857) because they were added to the interview after data collection was underway, and they are therefore ineligible for inclusion in symptom groupings (Any neurologic/CNS/PNS).

<sup>4</sup>No effect estimate presented for seizure due to lack of model convergence.

Figure 12. Association between ambient styrene concentration and neurologic symptom clusters adjusted for co-exposures (N=21,962).



Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, smoking status at enrollment, and total ambient coexposure concentration (separate models for each coexposure).

Ambient styrene exposure quartiles are based on National Air Toxics Assessment (NATA) 2011 estimates of annual average concentrations at the census tract level; referent exposure group is the lowest quartile (not shown on figure).

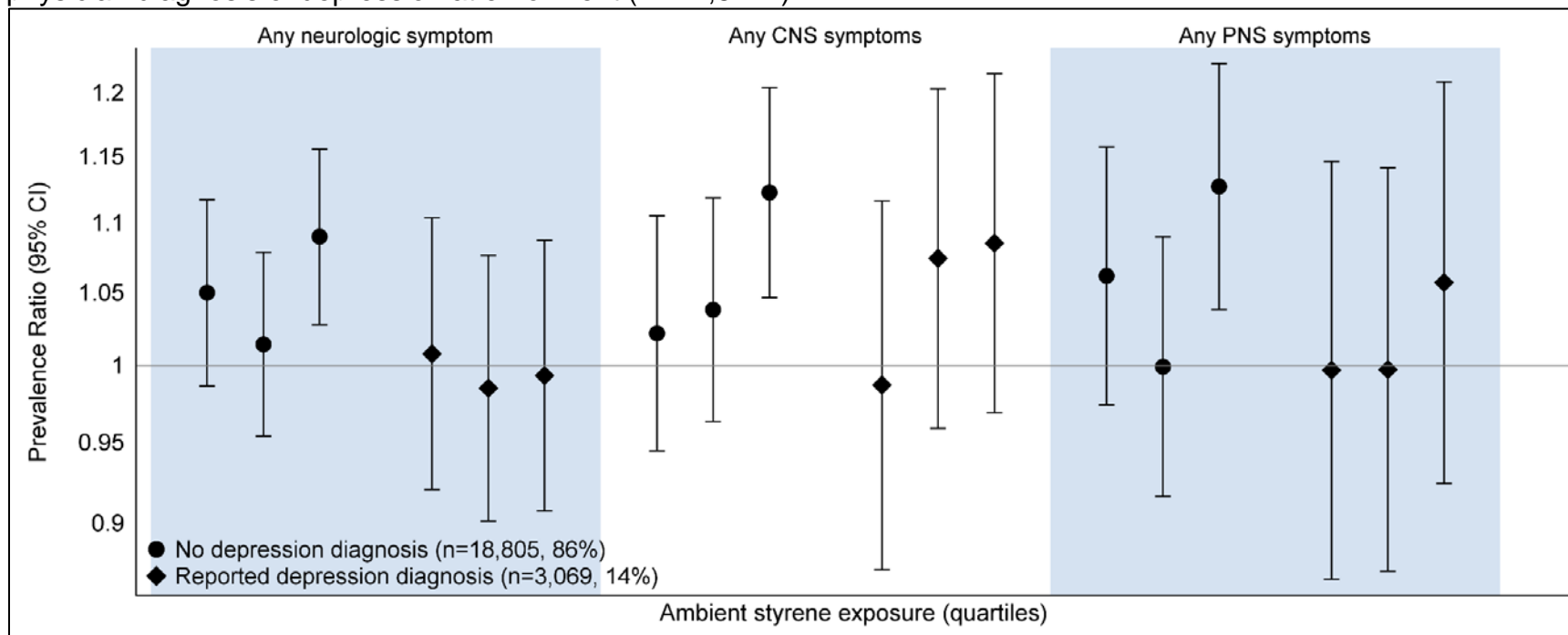
Central Nervous System (CNS) symptoms include: dizziness, headache, nausea, sweating, palpitations.

Peripheral Nervous System (PNS) symptoms include: tingling/numbness, blurred vision, stumbling.

Benzene and Toluene coexposures are NATA 2011 annual average census tract estimates of the total concentration.

Particulate matter with a diameter less than 2.5 micrometers (PM 2.5) coexposures are annual averages of US EPA Fused Downscaler Model daily census tract estimates.

Figure 13. Association between ambient styrene concentration and neurologic symptom clusters stratified by self-reported physician diagnosis of depression at enrollment (N=21,8741).



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Models adjusted for sex, age, season, race, employment status at enrollment, drinking status at enrollment, and smoking status at enrollment.

Central Nervous System (CNS) symptoms include: dizziness, headache, nausea, sweating, palpitations.

Peripheral Nervous System (PNS) symptoms include: tingling/numbness, blurred vision, stumbling.

Ambient styrene exposure quartiles are based on National Air Toxics Assessment (NATA) 2011 estimates of annual average concentrations at the census tract level; referent exposure group is the lowest quartile (not shown on figure).

Depression was ascertained via self-report: participants reported whether they had ever received a diagnosis of depression from a doctor.

<sup>1</sup>Excludes 88 participants missing information on self-reported depression diagnosis at enrollment.

## **CHAPTER VI: STYRENE EXPOSURE AND PERIPHERAL NEUROLOGIC FUNCTION (SPECIFIC AIM 3)**

### **A. Introduction**

Styrene is a hydrocarbon used in plastics, fiberglass, rubber, and resins. It is used to manufacture products such as insulation, fiberglass boats, automotive parts, car tires, Styrofoam, and plastic drinking glasses [1]. After the disposal of styrene-based products, styrene is released primarily into air, though smaller amounts are detected in soil and water [18]. Ambient styrene, a volatile organic compound, breaks down in the atmosphere within 1 to 2 days [1].

The general population is exposed to styrene primarily through inhalation of tobacco smoke, off-gassing of building materials, and vehicle and industrial emissions [2, 3]. The principal route of styrene exposure is through inhalation of contaminated air [1]. Typically, indoor air contains higher styrene levels than outdoor air [51] due to emissions from building materials, consumer products, and tobacco smoke [24].

Emissions from industrial activities and motor vehicle exhaust are the primary sources of styrene in outdoor air. The Gulf States are home to over half of all U.S. styrene production [1, 4], as well as many industrial and manufacturing facilities that use and emit styrene in the production of plastics, rubber, and fiberglass. This geographic clustering of industries potentially exposes Gulf residents to disproportionately high environmental styrene emissions from petrochemical, manufacturing, and coastal fishing and boating operations [21].



The half-life of styrene in blood is approximately 13 hours [5], so blood styrene levels reflect recent exposure. Approximately 40% of the U.S. adult population has measurable levels of styrene in their blood [24, 62].

Styrene is an established neurotoxicant at occupational exposure levels. Acute exposure causes depression of the central nervous system (CNS) with anesthesia-like properties at high exposure levels [1, 5, 6, 100]. Epidemiologic studies to date have focused on highly exposed workers, whose average blood levels were 25 times higher than those of the general population [7-14]. Occupational studies demonstrate styrene-induced neurotoxicity, evident as central (CNS) and peripheral nervous system (PNS) effects, from both acute and chronic inhaled exposure among highly-exposed workers. Acute effects include feeling “drunk” and tiredness [41], whereas impaired vision [99, 102], vestibular dysfunction [12], headaches [103], delayed reaction time [104, 105], impaired attention and memory [9], diminished hearing [106] and nerve conduction velocity [9, 107-110], and abnormal electroencephalogram results [110, 111] are likely persistent, chronic effects. These effects have been observed at lower occupational exposure levels in many [10, 99, 103-105, 114, 117-119], but not all [13, 120], studies.

Low-level, chronic styrene exposure may impact neurologic function at environmental levels relevant to the general population [20]. We investigated associations between environmental styrene exposure, assessed as both blood and air concentrations, and peripheral neurologic function.

## B. Methods

### Study Design and Participants

The Gulf Long-term Follow-up Study (GuLF STUDY) is a prospective cohort of adults (ages 21 and older) who participated in oil spill response activities and others who received safety training, but were not hired, following the *Deepwater Horizon* disaster [167]. Participants enrolled in the GuLF STUDY between March 2011 and March 2013.

A convenience sample of GuLF STUDY participants (N=1,055) living in the Gulf region enrolled in a Chemical Biomonitoring Study (CBS) between May 2012-July 2013. Participation in CBS involved providing an extra blood sample for measuring styrene and other compounds and completing a questionnaire about usual and recent exposures. Because styrene is rapidly cleared from the body and blood measurements were obtained two to three years after the oil spill, these levels represent usual, ongoing exposures (i.e., they are not due to oil spill cleanup work).

Another subset of GuLF STUDY participants (N=3,403) residing within approximately 60 miles of study clinics in New Orleans, Louisiana and Mobile, Alabama were recruited for a follow-up clinical examination between August 2014 and July 2016. Participation involved completing a clinical examination in one of two clinical settings (one in New Orleans, LA and one in Mobile, AL). Examinations included anthropometric measurements, biological sample collection, standardized computer-assisted neurocognitive testing, peripheral neurologic function evaluations, pulmonary function testing, and interviews on mental health and other factors.

Of the 3,403 participants who completed the follow-up clinical exam, 3,329 reported addresses at enrollment that were successfully geocoded to a 2010 U.S. Census tract. From this sample of participants with known residential locations, we excluded anyone with missing outcome information for all five neurologic function tests (n=8), missing demographic information (n=37), or missing covariate information (n=22). Of the remaining 3,262 participants with complete exposure, outcome, and covariate information, we restricted analyses to participants with no self-reported physician diagnosis of diabetes (i.e., we excluded 302 diabetics and 3 participants missing diagnosis information) because peripheral neuropathy is a known complication of diabetes [188]. This resulted in a final analytic sample of 2,956.

A total of 348 CBS participants with blood samples sufficient for quantification of styrene concentration also participated in the follow-up clinical examination. Of those, we excluded five participants missing all peripheral neurologic function information. The remaining 343 participants had complete exposure, outcome, demographic, and covariate information. We then excluded known diabetics (n=33), for a final analytic sample of 310.

Participants provided written consent and the Institutional Review Board of the National Institute of Environmental Health Sciences approved this study.

### **National Air Toxics Assessment**

The United States Environmental Protection Agency (EPA) 2011 National-scale Air Toxics Assessment (NATA) [189] estimates annual average ambient air toxic concentrations for each U.S. census tract. NATA evaluates 180 air toxics across the

United States using emissions inventories, dispersion, photochemical, and exposure modeling, and toxicity analyses. We used NATA styrene estimates to indicate typical, long-term environmental exposure by geocoding self-reported home addresses at enrollment, mapping each participant's location, and matching them to a corresponding 2010 U.S. census tract. The 2011 NATA annual average ambient styrene concentration corresponding to an individual's home census tract was applied as the estimate of usual ambient styrene exposure.

### **Blood styrene measurement**

We collected 10 mL of blood for styrene measurement using tubes containing potassium oxalate and sodium fluoride anticoagulant; tubes and stoppers were pre-treated by the Centers for Disease Control and Prevention (CDC) laboratory to remove VOCs to minimize pre-collection contamination [175, 176]. Samples were stored at 4°C until being shipped overnight on cold packs in biweekly batches to the Division of Laboratory Sciences, National Center for Environmental Health, CDC in Atlanta, Georgia for analysis of VOCs. Styrene was analyzed using equilibrium headspace solid-phase micro-extraction with benchtop gas chromatography/mass spectrometry following standard CDC procedures [168, 170].

### **Peripheral neurologic function testing**

The neurologic function testing battery, which was used in an earlier study of chronic, low-level neurotoxicants [148], included tests of visual acuity, visual contrast sensitivity, handgrip strength, vibrotactile threshold, standing steadiness, and single leg stance. These tests evaluate neurologic functions corresponding to neurotoxic effects

that have been demonstrated in association with occupational solvent exposure [149, 150].

**Visual Acuity**, an indicator of visual sharpness or clarity, was assessed using a standard vision testing instrument, the Optec 1000 (Optec, Inc. U.S.A). Results from this test were used to determine eligibility for analyses of visual contrast sensitivity.

**Visual contrast sensitivity** was evaluated with the Optec 1000 Functional Assessment of Contrast Sensitivity test. Circular stimuli consisting of alternating light and dark bars were presented. Nine stimuli of decreasing contrast were presented at each of 5 spatial frequencies (1.5, 3, 6, 12, and 18 cycles per degree). The weakest contrast correctly identified was recorded for each spatial frequency. Participants with visual acuity scores of 20/50 or worse were excluded from visual contrast sensitivity analyses.

**Standing steadiness** was evaluated with the Advanced Mechanical Technology, Inc. (AMTI, Inc., USA) force platform. Participants were instructed to stand on the platform without moving. Standing steadiness was measured twice with participant eyes open and twice with participant eyes closed. The force platform and associated software capture and store the forces applied to the platform by the participant's feet during each trial. The force signals are processed and plotted as a time series of locations (path) of the participant's center of pressure. The mean sway speeds in millimeters/second obtained during the two eyes open and the two eyes closed trials were used for statistical analysis of standing steadiness.

**Single leg stance** was evaluated by asking the participant to stand on one leg and maintain upright balance for 30 seconds [207]. If the participant was unable to

maintain their upright balance for the entire 30 second test interval, then the procedure was repeated up to two additional times. Inability to maintain single leg balance was defined as a need for steadying by the examiner to prevent falling or participant inability to comply with instructions to stand on only one leg for the entire 30 second test interval (i.e., the participant placed the other foot on the ground to prevent falling). Examiners indicated whether, and on which attempt, the participant was able to maintain his/her balance for the entire 30 second test interval. We modeled the outcome for single leg stance as inability to maintain upright balance for 30 seconds.

Vibrotactile sensory acuity was evaluated using a portable Vibratron II electromechanical vibrometer at a frequency of 120 Hz (Physitemp, Inc., USA). Examiners manually controlled the delivered vibration amplitude, obtaining five vibration threshold values (three descending and two ascending values) for each great toe. After discarding the first value, the final **vibrotactile threshold** for each toe was the median obtained from the remaining four values, which was converted to log microns of peak to peak amplitude displacement for statistical analysis.

**Handgrip strength** was assessed with a baseline digital hydraulic hand dynamometer that records the maximum force exerted by the participant's whole-hand grip. Participants performed three grip strength measures for each hand. We used the bilateral mean of all six tests, measured in pounds, as the summary metric in statistical analyses.

## Statistical analysis

We used multivariate linear regression to estimate continuous differences in peripheral neurologic function per unit change in the exposure metrics. For ease of interpretation, higher values indicate better performance for all continuous outcomes (mean contrast sensitivity score, vibrotactile threshold, handgrip strength, and postural sway speed). To achieve this internal consistency, we multiplied the raw values for vibrotactile threshold and postural sway speed by negative one. For tests of contrast sensitivity, we compared styrene-exposed to unexposed participants and evaluated differences in adjusted mean scores (adjustment covariates reported below) between exposure groups at each spatial frequency. We used log binomial regression to estimate prevalence ratios and corresponding 95% confidence intervals (PR, 95% CI) for single leg stance, the only dichotomous outcome. PRs above one indicate poorer test performance (i.e., inability to maintain balance for the full 30 seconds) and PRs below one indicate better test performance.

We analyzed each exposure type (i.e., NATA-estimated ambient styrene concentration and measured blood styrene concentration) separately using identical statistical methods for both. For associations with ambient styrene (N=2,956), exposure was categorized in quartiles, with the lowest quartile designated as the referent group. For analyses of measured blood styrene (n=310), exposure was dichotomized at the 90<sup>th</sup> percentile of the distribution, defining the top 10% of blood measurements as exposed and the remaining 90% as 'unexposed'. For all tests except visual contrast sensitivity, we additionally modeled blood styrene as the top quartile (exposed)

compared to the three lower quartiles ('unexposed'). We used all measured blood styrene values, including the actual values below the limit of detection [180].

All models were adjusted for gender (female vs male), age (continuous years), race (white, black, other), education (less than high school/equivalent, high school/general equivalency diploma, some college/two-year degree, four-year college graduate or more), employment status (working vs not working), alcohol consumption (current vs former/never), and smoking status (current vs former/never). Covariate information was obtained during the enrollment interview, and adjustment covariates were selected based on directed acyclic graph analysis [190] of the theoretical relationship between styrene exposure and peripheral neurologic function. For analyses of vibrotactile threshold and handgrip strength, we additionally adjusted for height. For visual contrast sensitivity, we restricted analyses to participants with better than 20/50 visual acuity and additionally adjusted for use of vision correction (i.e., wearing glasses or corrective lenses).

We examined a range of ambient and blood exposure contrasts in sensitivity analyses. Such additional ambient exposure assessments included dividing the top quartile of exposure at different values to assess variability at the upper end of the distribution, removing the third quartile and creating a referent group of values below the median to draw a sharper contrast between exposed and unexposed participants, and other combinations designed to maximize exposure contrasts. For blood styrene analyses, we used a similar approach, although the smaller sample size and statistical power limited analyses to dichotomous comparisons. Specifically, we evaluated the top 10%, top 15%, and highest quartile compared to participants below the median. We



also evaluated examiner and site effects by adjusting for examiner, and separately adjusting for and stratifying by clinic. Owing to concerns about overwhelming population variance in the handgrip strength tests, we also examined the influence of weight and gender on these associations.

Because eligibility criteria required that participants completed at least one peripheral neurologic function test from the full battery (but not all such tests), analytic sample sizes vary between tests.

All statistical analyses were conducted in SAS 9.4 (Cary, NC, USA).

### **C. Results**

Participants in the ambient analysis and CBS were largely similar with respect to demographic characteristics (Table 9). Approximately 70% of participants completed their examinations at the Alabama testing site, less than half attended college, three-fourths were male, and about half were younger than age 45 years. Half of all participants were white and about 40% were black. Among CBS participants, 85% completed all five peripheral neurologic function tests, compared to 81% of those in ambient analyses. Participants who were excluded due to incomplete covariate information are similar to participants included in the analytic sample with respect to the demographic characteristics reported in Table 9. We provide descriptive statistics on neurologic function in this population, presented as mean outcomes by exposure status and demographic group, in Tables 10 and 11, respectively.

For ambient exposure analyses, we modeled associations for each quartile of styrene exposure compared to the lowest quartile ( $\leq 0.01 \mu\text{g}/\text{m}^3$ ). Styrene

concentrations in the highest quartile ranged from 0.03 to 0.27  $\mu\text{g}/\text{m}^3$ , with most measurements below 0.05  $\mu\text{g}/\text{m}^3$  (Figure 14). For analyses of blood styrene, we prioritized a binary exposure divided at the 90<sup>th</sup> percentile, 0.83 ng/mL. The maximum observed concentration was 3.03 ng/mL, with most participants having blood levels below 1.36 ng/mL. CBS participants have measured blood styrene levels 2-3 times higher than those measured in the National Health and Nutrition Examination Survey [172], but an order of magnitude below typical occupational blood styrene levels. The blood exposure distribution demonstrated a pronounced right skew.

For tests of contrast sensitivity, we compared the highest quartile of ambient styrene to the lower two quartiles (n=1,692). Low exposure group participants performed better than those with high exposure at each spatial frequency, with significant ( $p < 0.05$ ) differences at 1.5, 3, and 12 cycles/degree (Figure 15). Results were virtually unchanged when we included the third quartile of ambient styrene in the referent group. We observed similar results for blood styrene above or below the 90<sup>th</sup> percentile (n=231), though the smaller sample size reduced precision and the only statistically significant difference was observed at 12 cycles/degree. Results were similar in sensitivity analyses unadjusted for vision correction, as well as those excluding participants with vision correction. These curves demonstrate a subtle, but consistent, decrement in visual contrast sensitivity associated with styrene exposure across spatial frequencies.

Increasing ambient styrene concentration was associated with reduced vibrotactile sensitivity (n=2,888) (Figure 16). We observed an exposure-response relationship ( $p=0.003$ ), with a significant effect in the highest exposure group ( $\beta=-0.13$

log microns; 95% CI: -0.23, -0.03) (Table 12). For blood styrene (n=307), we observed a non-significant, but suggestive, association between the highest quartile of exposure and reduced vibrotactile sensitivity (Figure 16, Table 12). When comparing, the top 10% to the lower 90% of blood styrene concentrations, this association was stronger ( $\beta$ =-0.39 log microns; 95% CI: -0.72, -0.05). For comparison, we found that aging ten years yielded a change in vibrotactile threshold of -0.49 log microns, compared to -0.39 log microns associated with a blood styrene measurement above the 90<sup>th</sup> percentile and -0.13 log microns for the highest quartile of ambient styrene.

Ambient styrene exposure (n=2,855) was associated with decreased standing steadiness, with statistically significantly worse stability at each quartile of exposure (Figure 17). The linear trend across quartiles of exposure was significant when participants' eyes were closed (p=0.02), as well as open (p < 0.0001). The highest quartile of exposure was associated with a difference in sway speed of 4.5 mm/s for both tests (eyes closed and open). For comparison, a ten-year difference in age was associated with differences in sway speed of 7.4 and 4.2 mm/s for eyes closed and open, respectively. Put alternatively, exposure-related effects were equivalent to 0.13 and 0.24 standard deviations of mean sway speed for eyes closed and open, respectively. In contrast, we did not observe effects in blood styrene exposure analyses (n=299).

Styrene exposure was also positively associated with impairments in single leg stance (Figure 18). Participants in the highest quartile of ambient styrene exposure were 40% more likely to experience balance loss during the test compared to those in the lowest quartile (PR, 1.42; 95% CI: 1.17, 1.71) and those with blood measurements

above the 90<sup>th</sup> percentile were twice as likely to lose their balance compared to all others (PR, 2.07; 95% CI: 1.18, 3.61). A sensitivity analysis comparing participants unable to maintain balance to those who maintained balance on the first attempt yielded similar, albeit slightly attenuated, results as the main analysis.

Unexpectedly, handgrip strength improved with increasing ambient styrene exposure (n=2,930), demonstrating a significant linear trend ( $p < 0.0001$ ). Higher exposure groups (i.e., the third and fourth quartile) were associated with increases of approximately six pounds of force (Figure 19). We did not observe associations between blood styrene exposure and handgrip strength (n=310).

Assessing other exposure contrasts revealed very slight changes in associations, with some subtle attenuation and loss in precision for ambient styrene exposure analyses. Overall, results were robust to a variety of exposure definitions and associations observed in the main analyses persisted. Exposure-related differences in neurologic function were less apparent and relatively imprecise for analyses of blood styrene and modeling exposure using different blood styrene cutpoints did not influence these results meaningfully.

Adjustment for clinical examiner did not meaningfully change results, nor did adjustment for clinical site. We did not stratify analyses by clinic because the imbalanced geographic distribution of participation resulted in imprecise effect estimates among participants attending the Louisiana clinic. Handgrip strength results were robust to adjustment for weight and BMI, as well as the exclusion of female participants. We observed no changes in associations for handgrip strength in sensitivity analyses.

## D. Discussion

Our study of styrene-induced neurotoxicity investigated environmental exposure levels in association with five measures of peripheral neurologic function. We observed styrene-associated impairment in visual, sensory, and postural function, though we did not detect evidence of voluntary motor system effects. Differences in neurologic performance were generally more consistent in relation to ambient styrene exposure, but we also observed associations with measured blood styrene concentration.

When comparing mean visual contrast sensitivity scores between exposure groups across spatial frequencies, we observed modest effects, some of which achieved statistical significance. Overall, however, a consistent pattern emerged, revealing decrements in visual contrast sensitivity associated with higher ambient and blood styrene concentration. Some investigations of occupational styrene exposure among fiberglass plastic workers have also reported diminished contrast sensitivity [149, 208], although others found no exposure-related effects [209, 210]. Occupational exposure to organic solvents, generally, has been shown to affect visual contrast sensitivity [211], but the occupational styrene literature has emphasized loss of color vision specifically [102, 192, 193, 208, 212].

Cutaneous vibrotactile threshold testing assesses the integrity of the entire somatosensory pathway, including the peripheral sensory nerves. Cutaneous vibratory stimuli are carried on large, heavily myelinated, sensory nerve fibers, which are believed to be more susceptible to both systemic and focal insult than small myelinated and unmyelinated fibers. Furthermore, longer nerve fibers such as those innervating the toe are more susceptible than shorter fibers innervating other anatomical sites. Many occupational and environmental hazards, including some organic solvents, are known to

affect these fibers. We observed impairments in vibrotactile threshold associated with blood styrene concentration, as well as a significant monotonic exposure-response trend across increasing quartiles of ambient styrene exposure. For both exposure types, significant effects were observed among participants in the highest exposure groups (i.e., top 10% in blood styrene, top quartile in ambient styrene). For comparison, the observed decrement in vibrotactile threshold associated with a ten-year age difference was greater than styrene-associated decrements, although the magnitude was comparable between a decade of aging and the highest blood styrene exposure. Although this specific endpoint has not been widely evaluated in the styrene literature, one study of workers exposed to both styrene and toluene found exposure-related adverse changes in somatosensory evoked potentials [109] and another study of painters documented similar effects [213]. Cutaneous vibrotactile threshold is an established, sensitive, and well validated test, which has been used to assess peripheral neurologic function in a variety of settings [214]. As such, our results are compelling evidence of styrene-associated decreases in somatosensory function.

Handgrip strength provides information about the functional integrity of the voluntary motor system from the motor cortex to peripheral skeletal muscles. Sub chronic styrene exposure induced impaired motor function in rats [129, 131], but we are not aware of such effects in humans. We observed a paradoxical association between ambient styrene exposure and grip strength, with increased styrene concentration associated with increased grip strength, although we observed no associations between blood styrene concentration and grip strength. We attribute these paradoxical findings, in part, to high population variance of this measure. Detecting associations in the

presence of such variance would require a strong exposure effect on the nerves being tested. Because longer nerves are typically more susceptible to adverse effects of toxic exposures than shorter nerves, effects in the expected direction may have been observed had we been able to measure motor strength of the lower extremities. Unfortunately, such testing was not feasible in the current study. Tests of great toe vibrotactile threshold and standing stability do assess the longest peripheral nerves and are likely more sensitive to subtle adverse effects. We attempted to account for some grip strength variance by adjusting for body mass index and physical activity, as well as by excluding women, however results were unchanged in these analyses. Another possible explanation for the paradoxical association is residual confounding by socioeconomic status. In our study, ambient exposure was estimated geographically, and potentially covaries with socioeconomic status. Indeed, a recent analysis of NATA 2011 data reported that census tracts with greater proportions of nonwhite and low income populations are exposed to higher concentrations of ambient air toxics than their wealthier, less diverse counterparts [215]. In addition to potentially experiencing higher exposures, people living in communities characterized by lower socioeconomic status may also be more likely to engage in labor-intensive jobs or activities, and therefore be stronger as a consequence of occupational physical conditioning. As such, associations between ambient styrene and handgrip strength may be confounded by social factors, or simply reflect muscular training effects, as opposed to effects on motor control systems.

Tests of balance assess the integrated function of several components of the nervous system, including the vestibular apparatus, cerebellum, and proprioceptive

system [216]. Loss of functional integrity of any of these systems secondary to toxic exposure may affect postural stability. In the current study, we observed significant associations between all quartiles of ambient styrene exposure and decreased standing steadiness (i.e., increased sway), with a monotonic exposure response. We did not, however, detect any associations between postural sway and blood styrene concentration. Results for ambient styrene and inability to maintain single leg stance were similar to those observed for postural sway. We also observed an association between inability to maintain single leg balance and the top 10% of blood styrene exposure. Combined, these results suggest a potential impairment of the motor control system necessary to balance due to environmental styrene exposure. These findings are supported by a study of reinforced plastic boat builders, which also reported impaired postural stability in association with styrene exposure [12].

Although the occupational styrene literature has focused on central nervous system toxicity [1], the peripheral nervous system may be another critical target for styrene's neurotoxic effects, particularly at environmental levels. The peripheral nervous system is composed of multiple nerve fiber populations, including long, large diameter, heavily myelinated neurons with limited tolerance to physiological and toxicological insult. Therefore, portions of the peripheral nervous system may be more vulnerable to small perturbations, resulting in impaired peripheral nerve performance earlier, and at lower exposure levels, than other neurologic tissues.

A dopaminergic mechanism for styrene neurotoxicity remains the leading mechanistic hypothesis, although it is speculative [71]. Studies suggest that styrene exposure decreases dopamine levels and increases dopamine receptors in rodents and



humans [126-128]. In blood samples of styrene-exposed plastics workers, prolactin levels were elevated, indicating reduced prolactin inhibition by dopamine [198]. Consistent with disturbance of the dopaminergic functions of the brain, styrene exposure potentiates a dose-dependent decrease in brain dopamine in male rats [128], as well as cell loss and dopamine depletion in retinas isolated from female rats [132]. This finding supports the established association between occupational styrene exposure and impaired vision [133].

An important strength of the present study was the use of well-validated, quantitative measures of peripheral neurologic function which may detect toxicologic effects earlier in disease progression than clinical assessments [216, 217]. We used a broad test battery, assessing multiple domains of neurologic function in a clinical research setting. Further, we collected these quantitative measures of neurologic health outcome from among over 3,000 participants, providing sufficient statistical power to detect small differences in neurologic function relevant to the observed exposure range. Our study also investigated an understudied, diverse population, for whom we had detailed covariate, exposure, and outcome information.

Associations were not entirely consistent between blood and ambient exposure metrics, which may be attributable to the unique limitations of biomarkers and proxy exposures, or the temporal windows they reflect. Biomarkers of exposure potentially introduce confounding bias, whereas ambient proxy estimates may be subject to measurement error and exposure misclassification [200]. Our study attempted to address this tradeoff by assessing both types of exposure metrics. We used NATA estimates to represent typical, long-term environmental exposure, and blood styrene

measurements to capture internal exposure burdens resulting from recent exposures. NATA estimates have previously been used as indicators of air pollution exposure in epidemiologic studies of autism spectrum disorder [122, 123, 206] and neurodegenerative diseases [124]. Blood styrene is a validated biomarker specific to styrene exposure [74] and it has been used extensively in occupational research [6], as well as in general population monitoring [1].

In the Relationships of Indoor, Outdoor, and Personal Air (RIOPA) study, blood styrene demonstrated stronger correlation with indoor than outdoor air concentrations, but outdoor sources explained more variability in levels than did indoor sources [61, 75]. Indeed, we observed a lack of concordance between measured blood styrene and NATA estimated annual average concentrations. At low occupational styrene levels measured in air, blood, and urine, blood demonstrated the highest correlations with ambient exposures [45].

Our study was potentially limited by the cross-sectional design, and subsequent inability to establish temporality between styrene exposure and peripheral neurologic effects. Although results are unlikely to have been substantially biased by the limitations of cross-sectional study design, we were not able to discern between transient and persistent neurologic effects of styrene exposure. Additionally, we obtained only a single blood measurement for each individual, whereas repeated measures would potentially improve estimation of *usual* exposure. Blood styrene concentrations were obtained two to four years prior to neurologic testing, and may introduce exposure misclassification depending on the relevant timing of exposure.

Covariate information was obtained at enrollment or the time of the blood draw, and may not be reflective of immediate confounding exposures near the time of the peripheral neurologic testing. As such, the degree to which potential confounders changed between exposure and outcome ascertainment may have introduced residual confounding.

Using an annual average ambient concentration limits interpretation. However, as routine monitoring of ambient styrene in the U.S. doesn't provide sufficient temporal or spatial coverage to support exposure interpolation methods [143, 145, 155], NATA remains the only spatially-referenced exposure data source with sufficient geographic coverage for our study region. Although the assumptions inherent to an annual average estimate of air pollution potentially limit interpretation, NATA data are a valid estimation of usual exposure levels experienced by Gulf region residents. Long-term blood and ambient styrene trends indicate that year-to-year regional variation in concentration is generally not substantial, suggesting that an annual average estimate is an appropriate assessment of exposure [20, 60, 66, 74]. If long-term styrene exposure exerts chronic neurologic effects, NATA estimates may be a more relevant exposure metric than measures with more precise temporal windows.

Although styrene-associated impairment of peripheral neurologic function was not universally apparent across all exposure and outcome measures, in aggregate, our results are suggestive of peripheral neurotoxic effect of styrene. Particularly compelling are associations demonstrated with the standing steadiness and vibrotactile threshold tests, which are well-established and validated measures of peripheral neurologic performance with known standard covariates [217-219]. Testing peripheral neurologic

function in this way may allow for early detection of subclinical neurotoxicity among groups exposed at levels relevant to the general population. Furthermore, large fiber function abnormality may be an early indicator of peripheral neurologic disease. Future research is needed to confirm these findings and to determine whether styrene-induced neurotoxicity derived from environmental exposures persists over time, and how it may relate to future risk of clinically-apparent neurologic disease.

### **E. Conclusions**

Though styrene-associated impairment of peripheral neurologic function was not universally apparent across all exposure and outcome measures, our results taken together indicate a possible peripheral neurotoxic effect of styrene. Particularly compelling are associations demonstrated in the standing steadiness and vibrotactile threshold tests, which are regarded to be gold standard assessments, featuring well established operating characteristics, low noise, and known standard covariates [217-219]. Testing large fiber function in this way may allow for early detection of subclinical neurotoxicity at exposure levels relevant to the general population. Furthermore, large fiber function abnormality is a possible early indicator of peripheral neurological disease. Future research is needed to determine whether styrene-induced neurotoxicity derived from environmental exposures persists over time, and how it may relate to neurological disease.

## F. Tables and Figures

Table 9. Demographic characteristics of participants living in the Gulf States (N=2,956) and those participating in the Chemical Biomonitoring Study (CBS, N=310).

Characteristic		Ambient (n=2,956)		CBS (n=310)	
		N	%	N	%
Sex	Female	689	23.3	77	24.8
	Male	2,267	76.7	233	75.2
Age, years	< 30	463	15.7	59	19.0
	30 - 45	1,022	34.6	110	35.5
	< 45	1,471	49.8	141	45.5
Race	White	1,520	51.4	156	50.3
	Black	1,180	39.9	131	42.3
	Other	256	8.7	23	7.4
Work status	Employed	1,559	52.7	165	53.2
	Unemployed	1,397	47.3	145	46.8
Current drinker	Yes	2,132	72.1	215	69.4
	No	824	27.9	95	30.7
Current smoker	Yes	1,009	34.1	79	25.5
	No	1,947	65.9	231	74.5
Oil spill response work	≥ 1 day	2,518	85.2	275	88.7
	None	438	14.8	35	11.3
Education	< High school	625	21.1	66	21.3
	High school graduate	1,037	35.1	116	37.4
	Some college	873	29.5	92	29.7
	≥ College graduate	421	14.2	36	11.6
Body Mass Index, kg/m <sup>2</sup>	≤ Normal (< 25)	782	25.7	87	28.1
	Overweight (25 - < 30)	1,131	38.3	121	39.0
	Obese (≥ 30)	1,043	35.3	102	32.9
Clinic	Louisiana	904	30.6	87	28.1
	Alabama	2,052	69.4	223	71.9

Table 10. Mean outcomes by exposure status among modeled population.

Test	Units	Exposure	Ambient (n=2,956)			Exposure	Blood (n=310)		
			N	Mean	Std Dev		N	Mean	Std Dev
Grip strength	pounds	Q1	724	100.73	35.27	Lower 90	278	104.02	37.64
		Q2	738	101.75	35.90	Top 10	32	100.32	38.17
		Q3	745	108.65	40.03				
		Q4	723	108.76	39.13				
Vibrotactile threshold	log microns	Q1	707	1.85	1.21	Lower 90	275	1.71	1.19
		Q2	727	1.78	1.16	Top 10	32	1.82	1.06
		Q3	738	1.78	1.17				
		Q4	716	1.69	1.17				
Standing steadiness Closed eyes	mm/s	Q1	704	61.44	28.41	Lower 90	268	59.99	33.38
		Q2	721	63.09	40.32	Top 10	31	52.86	19.20
		Q3	731	61.48	36.37				
		Q4	699	57.52	27.60				
Standing steadiness Closed eyes	mm/s	Q1	704	39.15	13.58	Lower 90	268	37.99	14.73
		Q2	721	40.54	25.15	Top 10	31	37.76	12.19
		Q3	731	39.72	15.55				
		Q4	699	38.50	19.39				
Contrast sensitivity	score	Q1	672	5.03	1.03	Lower 90	263	5.02	1.02
		Q2	685	5.14	1.07	Top 10	30	4.65	1.07
		Q3	697	5.04	1.06				
		Q4	673	4.95	1.04				

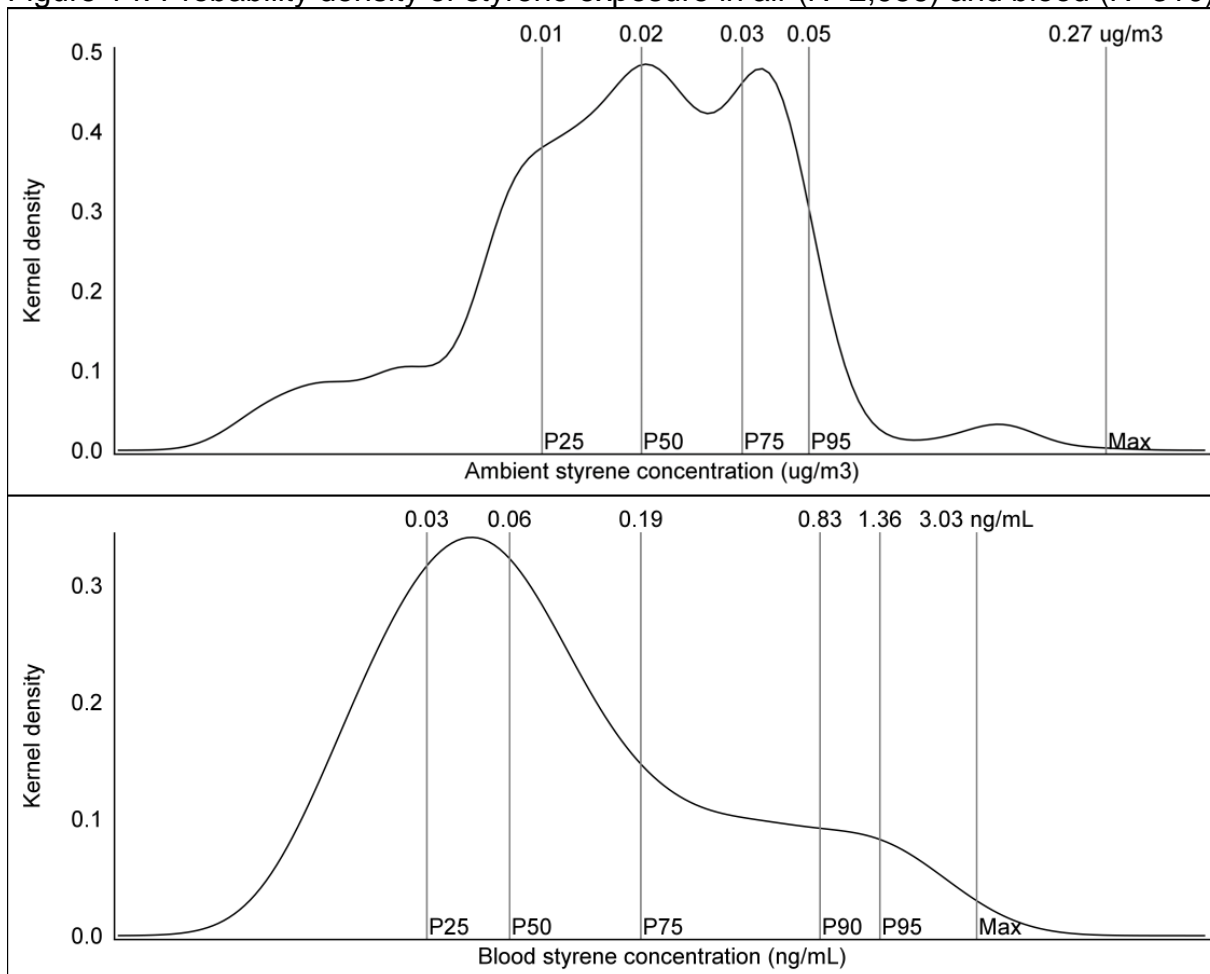
N, number; Std Dev, standard deviation of mean.

Table 11. Mean outcomes in eligible participant demographic subgroups.

		Grip strength <i>pounds</i>	Vibrotactile threshold <i>log microns</i>	Standing steadiness: closed <i>mm/s</i>	Standing steadiness: open <i>mm/s</i>	Contrast sensitivity <i>score</i>
Overall (n=3,262)	N	3234	3175	3143	3143	2987
	Mean	103.96	1.84	62.27	40.33	5.01
	Std Dev	37.55	1.20	36.49	20.10	1.06
Non-diabetics (n=2,956)	N	2930	2888	2855	2855	2727
	Mean	104.98	1.78	60.91	39.49	5.04
	Std Dev	37.81	1.18	33.73	18.96	1.05
Smokers (n=1,093)	N	1082	1067	1051	1051	983
	Mean	103.51	1.79	63.78	41.26	5.01
	Std Dev	35.84	1.15	41.06	23.11	1.04
Nonsmokers (n=2,169)	N	2152	2108	2092	2092	2004
	Mean	104.18	1.86	61.51	39.87	5.02
	Std Dev	38.39	1.22	33.95	18.39	1.07
Men (n=2,506)	N	2483	2434	2414	2414	2313
	Mean	113.15	1.95	65.48	41.67	5.09
	Std Dev	35.59	1.23	39.09	20.52	1.07
Women (n=756)	N	751	741	729	729	674
	Mean	73.55	1.49	51.65	35.91	4.76
	Std Dev	26.09	1.01	23.14	17.96	1.00

N, number; Std Dev, standard deviation of mean.

Figure 14. Probability density of styrene exposure in air (N=2,956) and blood (N=310).

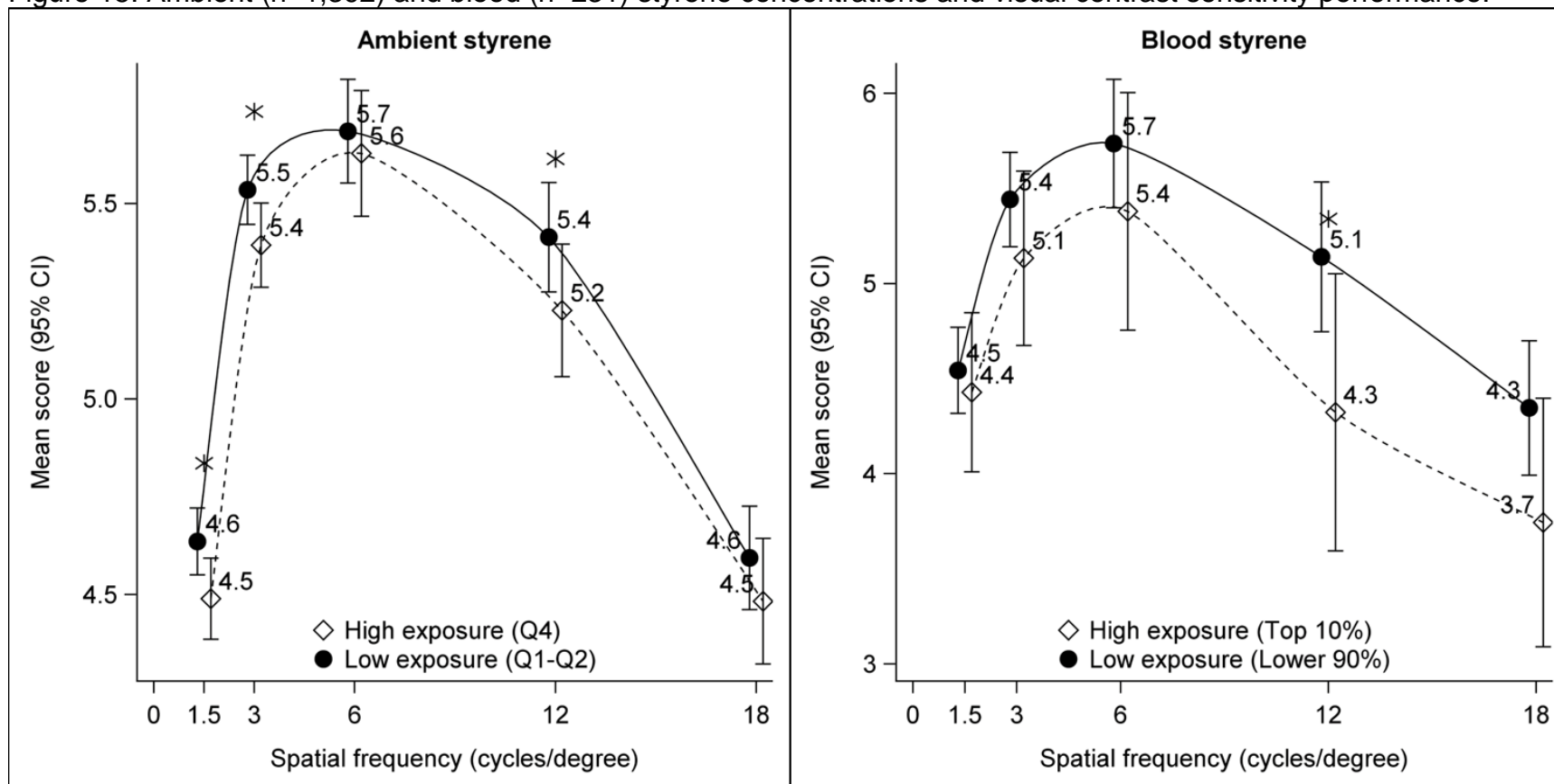


Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average concentrations ( $\mu\text{g}/\text{m}^3$ ) at the census tract level. Blood styrene exposure concentrations ( $\text{ng}/\text{mL}$ ) are measured from a single blood draw obtained in the participant's home.

Values at the top of reference lines indicate exposure concentrations; labels at the bottom of reference lines indicate locations in the exposure distribution: P25, 25<sup>th</sup> percentile; P50, 50<sup>th</sup> percentile; P75, 75<sup>th</sup> percentile; P90, 90<sup>th</sup> percentile; P95, 95<sup>th</sup> percentile; Max, maximum value.



Figure 15. Ambient (n=1,392) and blood (n=231) styrene concentrations and visual contrast sensitivity performance.



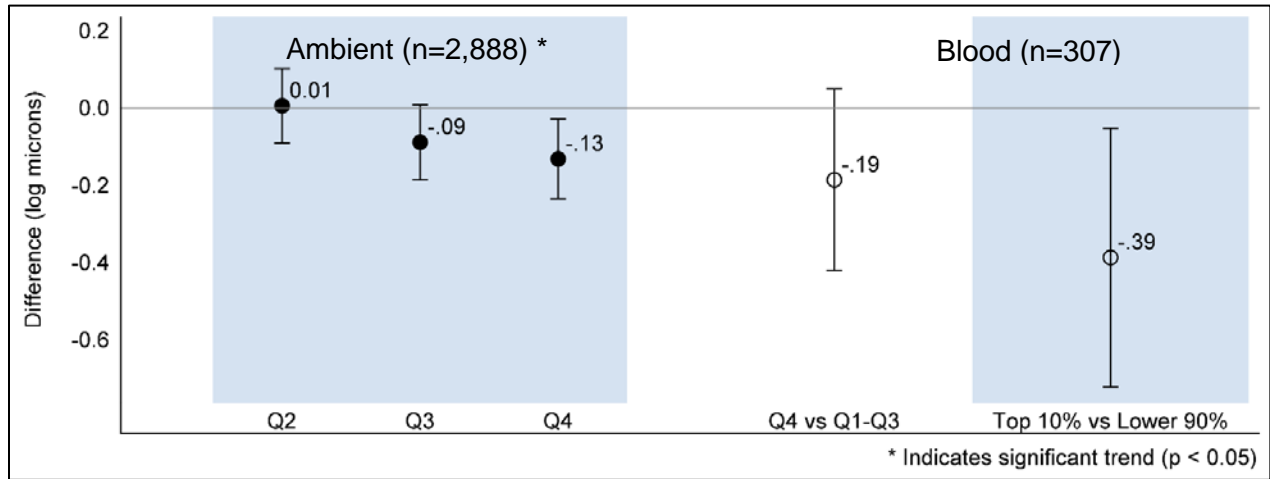
Symbol markers and labels indicate mean score at each spatial frequency; error bars indicate 95% confidence intervals associated with mean scores.

Models adjusted for vision correction (eyeglasses, corrective lenses), gender, age, race, enrollment employment status, enrollment drinking status, and enrollment smoking status. Excluded participants with 20/50 or worse visual acuity. Asterisks indicate p-value for difference in means < 0.05.

Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average census tract concentrations ( $\mu\text{g}/\text{m}^3$ ). Q4, fourth quartile; Q1-Q2, first and second quartile (below median).

Blood styrene exposure concentrations (ng/mL) are measured from a single blood draw obtained in the participant's home. High exposure, blood styrene measurements above the 90<sup>th</sup> percentile of the distribution; Low exposure, blood styrene measurements up to the 90<sup>th</sup> percentile of the distribution.

Figure 16. Ambient (n=2,888) and blood (n=307) styrene concentrations and differences in vibrotactile threshold.



Symbol markers and labels indicate change in vibrotactile threshold multiplied by -1 (log microns) associated with each exposure metric; negative values reflect neurologic deficits; positive values reflect neurologic improvement; diamond, age (decade); empty circles, blood styrene exposures; filled circles, ambient styrene quartiles; error bars indicate 95% confidence intervals associated with effect estimates.

Models adjusted for gender, age, height, race, enrollment employment status, enrollment drinking status, and enrollment smoking status.

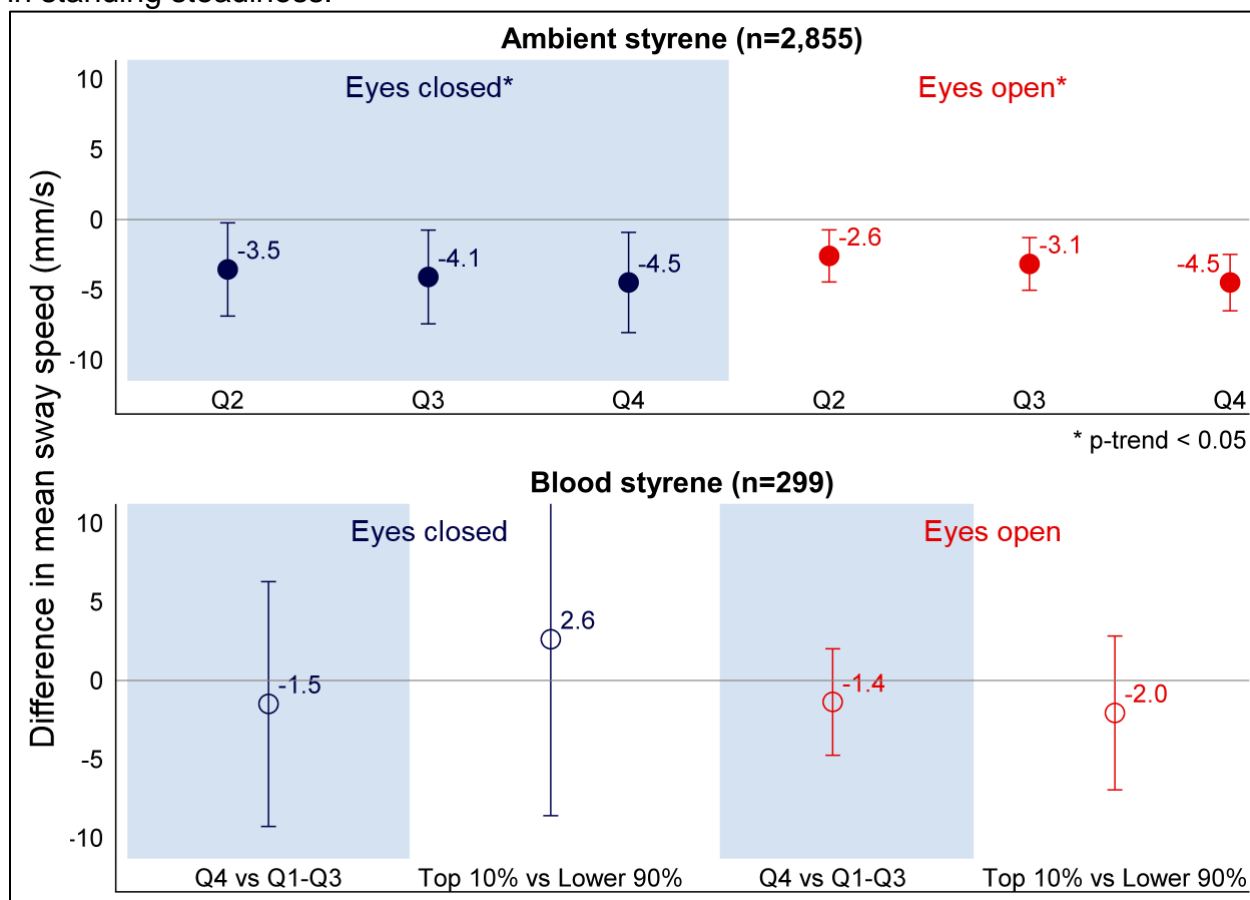
Asterisks indicate p-value for linear trend < 0.05.

Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average concentrations ( $\mu\text{g}/\text{m}^3$ ) at the census tract level.

Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.

Blood styrene exposure concentrations (ng/mL) are measured from a single blood draw obtained in the participant's home. Top 10%, blood styrene measurements above the 90<sup>th</sup> percentile of the distribution; Lower 90%, blood styrene measurements up to the 90<sup>th</sup> percentile of the distribution.

Figure 17. Ambient (n=2,855) and blood (n=299) styrene concentrations and differences in standing steadiness.



Symbol markers and labels indicate change in sway speed multiplied by -1 (mm/s) associated with each exposure metric; negative values reflect neurologic deficits; positive values reflect neurologic improvement; error bars indicate 95% confidence intervals associated with effect estimates.

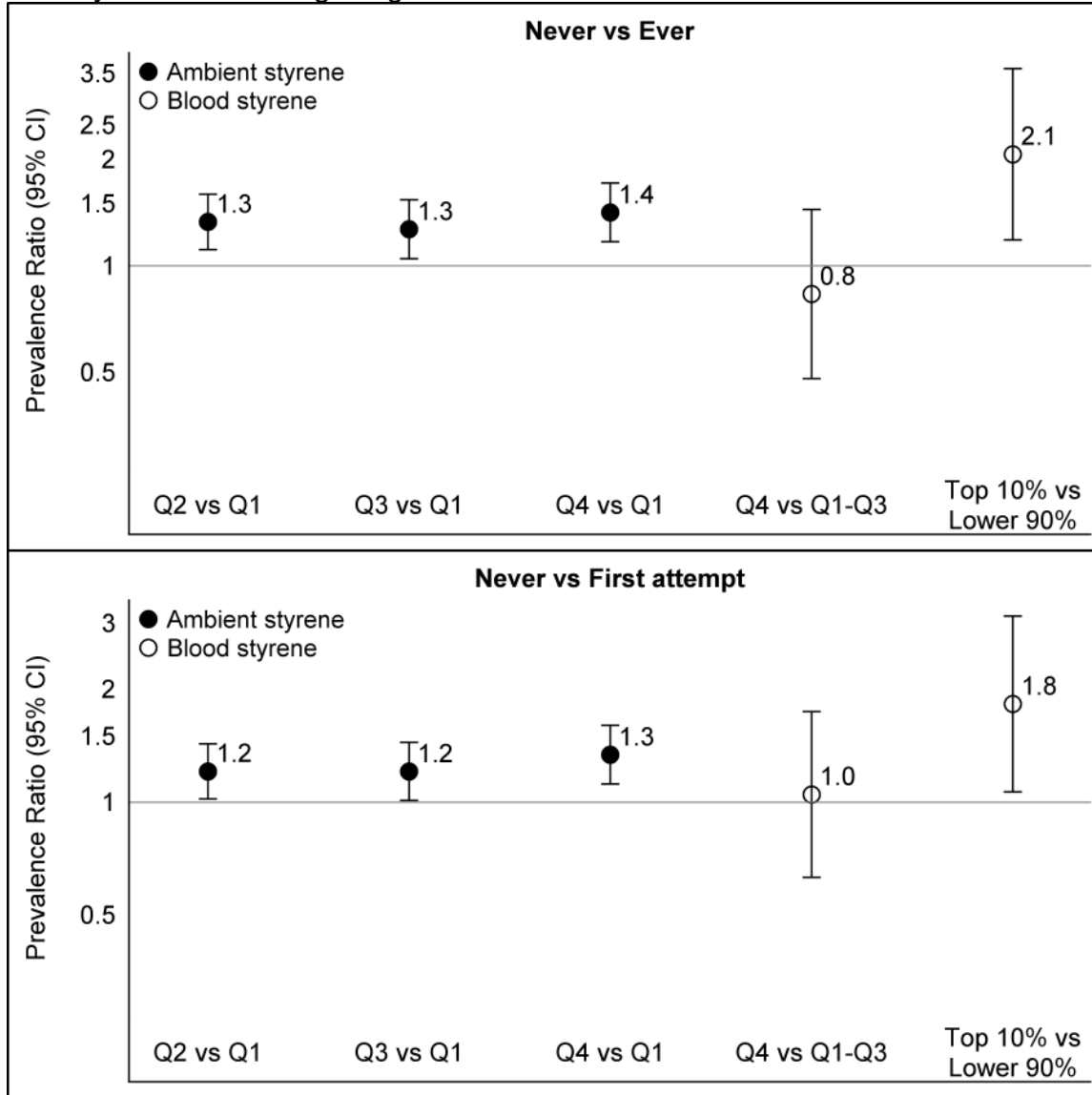
Models adjusted for gender, age, race, enrollment employment status, enrollment drinking status, and enrollment smoking status.

Asterisks indicate p-value for linear trend < 0.05.

Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average census tract concentrations ( $\mu\text{g}/\text{m}^3$ ). Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.

Blood styrene exposure concentrations (ng/mL) are measured from a single blood draw obtained in the participant's home. Top 10%, blood styrene measurements above the 90<sup>th</sup> percentile of the distribution; Lower 90%, blood styrene measurements up to the 90<sup>th</sup> percentile.

Figure 18. Ambient (n=2,271) and blood (n=237) styrene concentrations and inability to maintain single leg stance.



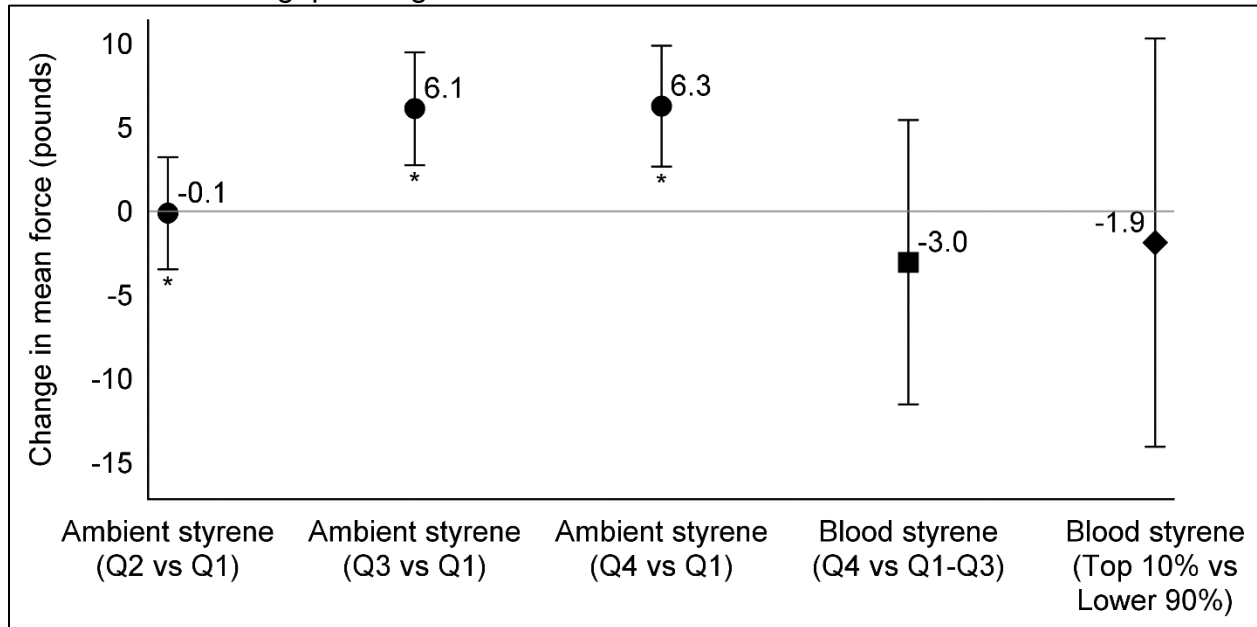
Prevalence ratio, inability to maintain balance for 30 seconds on any of three attempts compared to ability to maintain balance for 30 seconds on first attempt. Values above 1 indicate problems with balance; values below 1 indicate ability to balance for 30 seconds.

Error bars indicate 95% confidence intervals associated with prevalence ratios. Models adjusted for gender, age, race, enrollment employment status, enrollment drinking status, and enrollment smoking status.

Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average concentrations ( $\mu\text{g}/\text{m}^3$ ) at the census tract level. Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.

Blood styrene exposure concentrations (ng/mL) are measured from a single blood draw obtained in the participant's home. Top 10%, blood styrene measurements above the 90<sup>th</sup> percentile of the distribution; Lower 90%, blood styrene measurements up to the 90<sup>th</sup> percentile.

Figure 19. Ambient (n=2,930) and blood (n=310) styrene concentrations and differences in handgrip strength.



Symbol markers and labels indicate change in mean force (pounds) associated with each exposure metric; circles, ambient styrene quartiles; square, blood styrene highest quartile; diamond, blood styrene 90<sup>th</sup> percentile; error bars indicate 95% confidence intervals associated with effect estimates.

Models adjusted for gender, age, height, race, enrollment employment status, enrollment drinking status, and enrollment smoking status.

Asterisk indicate p-value for linear trend < 0.05.

Ambient styrene exposure is based on National Air Toxics Assessment (NATA) 2011 modeled estimates of annual average concentrations ( $\mu\text{g}/\text{m}^3$ ) at the census tract level. Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.

Blood styrene exposure concentrations (ng/mL) are measured from a single blood draw obtained in the participant's home. Top 10%, blood styrene measurements above the 90<sup>th</sup> percentile of the distribution; Lower 90%, blood styrene measurements up to the 90<sup>th</sup> percentile of the distribution.

Table 12. Associations between ambient (N=2,956) and blood (N=310) styrene concentrations and peripheral nerve function.

Peripheral neurologic function test	N	Exposure	Ambient Effect (95% CI)	p-trend	N	Exposure	Blood Effect (95% CI)	p-value
Vibrotactile threshold <sup>1</sup> <i>β, Change in log microns</i>	2,888	Q1	Ref	0.003	307	Q1-Q3	Ref	—
		Q2	0.02 (-0.07, 0.12)			Q4	-0.17 (-0.41, 0.06)	0.14
		Q3	-0.07 (-0.16, 0.03)			Low 90%	Ref	—
		Q4	-0.12 (-0.22, -0.01)			Top 10%	-0.37 (-0.71, -0.04)	0.03
Grip strength <i>β, Change in force (pounds)</i>	2,956	Q1	Ref	<0.0001	310	Q1-Q3	Ref	—
		Q2	-0.11 (-3.45, 3.23)			Q4	-3.04 (-11.52, 5.44)	0.48
		Q3	6.13 (2.76, 9.49)			Low 90%	Ref	—
		Q4	6.27 (2.67, 9.87)			Top 10%	-1.86 (-14.04, 10.31)	0.76
Standing steadiness, eyes closed <sup>1</sup> <i>β, Change in sway speed (mm/s)</i>	2,855	Q1	Ref	0.02	299	Q1-Q3	Ref	—
		Q2	-3.72 (-7.01, -0.43)			Q4	-1.54 (-9.27, 6.19)	0.70
		Q3	-4.31 (-7.62, -1.00)			Low 90%	Ref	—
		Q4	-4.65 (-8.20, -1.10)			Top 10%	-2.46 (-8.71, 13.64)	0.66
Standing steadiness, eyes open <sup>1</sup> <i>β, Change in sway speed (mm/s)</i>	2,855	Q1	Ref	<0.0001	299	Q1-Q3	Ref	—
		Q2	-2.68 (-4.53, -0.83)			Q4	-1.36 (-4.73, 2.01)	0.43
		Q3	-3.22 (-5.08, -1.36)			Low 90%	Ref	—
		Q4	-4.53 (-6.52, -2.54)			Top 10%	-2.09 (-6.97, 2.78)	0.40
Single leg stance, never vs ever <sup>2</sup> <i>PR, Failure to stand for 30 seconds</i>	2,821	Q1	Ref	0.001	295	Q1-Q3	Ref	—
		Q2	1.33 (1.11, 1.59)			Q4	0.82 (0.47, 1.42)	0.48
		Q3	1.26 (1.04, 1.52)			Low 90%	Ref	—
		Q4	1.40 (1.16, 1.70)			Top 10%	2.05 (1.20, 3.53)	0.01
Single leg stance, never vs 1st attempt <sup>3</sup> <i>PR, Failure to stand for 30 seconds</i>	2,271	Q1	Ref	0.002	237	Q1-Q3	Ref	—
		Q2	1.19 (1.00, 1.41)			Q4	1.07 (0.65, 1.76)	0.79
		Q3	1.19 (1.00, 1.442)			Low 90%	Ref	—
		Q4	1.33 (1.11, 1.59)			Top 10%	1.82 (1.07, 3.08)	0.03



	N	Range	Effect (95% CI)	p-value	N	Range	Effect (95% CI)	p-value
Contrast sensitivity <sup>4</sup>	2,956	1.5	0.15 (0.04, 0.25)	0.01	231	1.5	0.12 (-0.29, 0.52)	0.56
<i>Difference, mean contrast score</i>		3	0.14 (0.03, 0.25)	0.01		3	0.30 (-0.14, 0.75)	0.18
		6	0.05 (-0.11, 0.21)	0.53		6	0.34 (-0.26, 0.95)	0.27
		12	0.18 (0.01, 0.35)	0.04		12	0.79 (0.08, 1.50)	0.03
		18	0.10 (-0.06, 0.26)	0.23		18	0.58 (-0.05, 1.22)	0.07

<sup>1</sup>Reported values for these tests are raw values multiplied by -1, so that negative numbers indicate diminished neurologic function and positive numbers indicate improved neurologic function for all continuous outcomes.

<sup>2</sup>Single leg stance prevalence ratio = inability to maintain balance for 30 seconds on any of three attempts / ability to maintain balance for 30 seconds on any of three attempts. Values above 1 indicate diminished balance, values below 1 indicate improved balance.

<sup>3</sup>Single leg stance prevalence ratio = inability to maintain balance for 30 seconds on any of three attempts / ability to maintain balance for 30 seconds on first attempt. Values above 1 indicate diminished balance, values below 1 indicate improved balance.

<sup>4</sup>For contrast sensitivity: ambient exposure contrast is Q4 vs Q1-Q2; blood exposure contrast is Top 10% vs Lower 90%; restricted to participants with normal visual acuity (better than 20/50).

## CHAPTER VII: CONCLUSIONS

### A. Summary of results

Styrene is an established neurotoxicant, though epidemiologic research to date has focused on occupational exposure levels and emphasized central nervous system effects. In this dissertation, we evaluated sources of environmental styrene exposure in Gulf coast residents, and examined associations of such exposure levels with neurologic effects. We included in our assessment of neurologic effects a detailed assessment of peripheral neurologic function.

For Specific Aims 1 and 2, we evaluated personal, environmental, and spatial predictors as determinants of blood styrene levels in a population of Gulf coast residents with apparently elevated environmental styrene exposure. Blood styrene levels in this population were two to three times higher than those observed in NHANES, for smokers and nonsmokers, with marked differences at the upper end of the distribution. Indeed, nonsmokers from our study sample were 5.9 (95% CI: 5.0, 6.8) times as likely as NHANES participants to have blood styrene levels exceeding the NHANES 95<sup>th</sup> percentile. Smokers from the Gulf coast population were 4.3 (95% CI: 3.5, 5.1) times as likely as their NHANES counterparts to have elevated levels.

Our predictive model of determinants of styrene exposure captured only 20% of the variability in blood levels, and results were largely confirmatory with previous literature on VOC exposures. Smoking ( $\beta = 0.42$  log ng/mL, 95% CI: 0.34, 0.51), vehicular emissions ( $\beta = 0.34$ , 95% CI: 0.09, 0.59), time spent on boats ( $\beta = 1.10$ , 95% CI: 0.31, 1.89), mobile housing ( $\beta = 0.35$ , 95% CI: 0.07, 0.63), and home exterior

materials ( $\beta = 0.37$ , 95% CI: 0.11, 0.64) were associated with increases in blood styrene. In primary analyses, neither ambient styrene concentrations nor proximity to point source emissions of styrene predicted blood styrene levels. In a subgroup defined to more efficiently examine environmental sources, however, we did observe associations between elevated blood styrene and ambient styrene due to nonpoint sources. The subgroup was defined as participants with fall/winter blood draws, no reported recent active or passive tobacco smoke exposure, and not living in Alabama. Participants in this subgroup with the highest quartile of ambient nonpoint styrene exposure were 2.1 (95% CI: 1.0, 4.4) times as likely as participants in the lowest exposure quartile to have blood styrene measurements exceeding the NHANES 95<sup>th</sup> percentile. As in the main analysis, proximity to point source styrene emissions was not an informative predictor in any subgroup analyses.

For Specific Aim 3, we evaluated cross-sectional associations between styrene exposure and neurologic symptoms. We considered both ambient and blood styrene in relation to each symptom individually, as well as clustered outcomes defined by the presence of any neurologic, CNS, or PNS symptom. We found that participants with the highest quartile of ambient styrene exposure (0.03-1.70  $\mu\text{g}/\text{m}^3$ ) had significantly increased prevalence of each symptom and cluster, when compared to participants in the lowest quartile of exposure ( $\leq 0.01 \mu\text{g}/\text{m}^3$ ). Furthermore, we observed significant exposure-response trends ( $p < 0.05$ ) across quartiles of exposure for all outcomes except nausea and fatigue. The highest quartile of exposure was associated with a 7% increase (PR = 1.07, 95% CI: 1.02, 1.12) in reporting at least one neurologic symptom, a 20% increase (PR = 1.20, 95% CI: 1.09, 1.32) in reporting multiple CNS symptoms,

and a 12% increase (PR = 1.12, 95% CI: 1.02, 1.23) in reporting multiple PNS symptoms. While effect sizes are small (suggesting subtle neurotoxic effects) and exposure levels are low, the consistency across symptoms and the exposure-response relationships suggest that these associations are not due to chance alone.

When evaluating blood styrene levels in relation to neurologic symptoms, relationships were less consistent. We did not observe increases in symptom clusters associated with blood styrene exposure above the median value (0.067 ng/mL), apart from a 40% increase among participants reporting multiple PNS symptoms (PR = 1.40, 95% CI: 1.00, 1.97). Suggestive associations were apparent for dizziness (PR = 1.36, 95% CI: 0.91, 2.04), nausea (PR = 1.53, 95% CI: 0.93, 2.53), and stumbling (PR = 2.14, 95% CI: 1.03, 4.44). The remaining symptoms did not appear to be related to blood styrene exposure. While the overall relationship between exposure and outcome is less consistent for blood styrene than for ambient exposure, the magnitude of the associations was larger where we did detect exposure-related effects in blood. These results lacked precision, due at least in part to the smaller sample size (N=874), and may be subject to greater misclassification given the short half-life of the exposure measure and the fact that symptom data were reported before the blood draw

As an extension of Specific Aim 3, we examined the effect of styrene exposure on peripheral neurologic function using a battery of objective neurologic tests. For primary analyses, we assessed associations between quartiles of ambient styrene exposure and five dimensions of neurologic function (visual contrast sensitivity, vibrotactile threshold, postural stability, single leg stance, and handgrip strength). Higher exposure was associated with impaired visual contrast sensitivity at all spatial

frequencies, though differences were subtle, and were only statistically significant at 1.5 (mean difference = 0.15, 95% CI: 0.04, 0.25), 3 (mean difference = 0.14, 95% CI: 0.03, 0.25), and 12 (mean difference = 0.18, 95% CI: 0.01, 0.35) cycles/degree. We observed a monotonic decrement in vibrotactile sensitivity with increasing ambient exposure (4<sup>th</sup> quartile,  $\beta$  = -0.12, 95% CI: -0.22, -0.01). All quartiles of ambient styrene exposure were associated with significant decreases in postural stability, with a statistically significant exposure-response relationship (eyes closed,  $p$  = 0.01; eyes open,  $p$  < 0.0001). We found similar evidence of vestibular dysfunction for single leg stance, with participants in the highest quartile demonstrating a 40% increase in inability to maintain balance (PR = 1.4, 95% CI: 1.2, 1.7). We observed a statistically significant, but paradoxical, association of improving handgrip strength with increasing ambient styrene exposure.

In secondary analyses for Specific Aim 3, we modeled associations between more extreme blood styrene exposure measures and the same five tests of peripheral neurologic function (n=310). We assessed exposure as the top 10% of blood levels (versus the lower 90%), as well as the top quartile (versus all other quartiles) of the distribution. Participants with blood styrene measures above the 90<sup>th</sup> percentile had slightly, but consistently, lower visual contrast sensitivity scores. The difference was significant at 12 cycles/degree only (mean difference = 0.79, 95% CI: 0.08, 1.50), but a similar pattern was evident at all spatial frequencies. Similarly, the top 10% of blood styrene exposure was associated with diminished performance on tests of vibrotactile threshold ( $\beta$  = -0.37 log microns, 95% CI: -0.71, -0.04) and single leg stance (PR = 2.1, 95% CI: 1.2, 3.5). We did not detect any exposure-related associations for tests of postural stability or handgrip strength. Further, the highest quartile of blood styrene

levels was not associated with meaningful differences in peripheral neurologic function on any of the tests.

Styrene exposure, both ambient concentrations and blood levels, was associated with self-reported PNS symptoms. Associations were stronger and more precise for participants reporting two or more PNS symptoms. This suggestion of a styrene-associated peripheral neurologic effect was corroborated in analyses of objective peripheral neurologic function testing where we observed suggestive associations between styrene exposure and vision, sensory, and vestibular impairment. We compare associations between symptoms and corresponding neurologic function tests cautiously, as the analyses are not perfectly exchangeable. Not only is the timing between exposure and outcome ascertainment different between analyses, but the subjective and objective outcomes are imperfect analogues of each other. Therefore, we draw the following comparisons speculatively.

Vision impairment was assessed two ways, as self-reported blurred vision and as visual contrast sensitivity score (Table 13). We observed associations between ambient styrene and both metrics, self-reported and objectively measured, vision impairment. Blood styrene exposure was associated with objectively measured vision impairment, but not with self-reported blurred vision. One explanation for the discrepancy in findings with blood styrene is that blurred vision might be expected to be an acute effect, whereas contrast sensitivity may be a more long-term measure. If this is the case, the timing of symptom ascertainment and the blood measurement would inhibit our ability to detect an association. Styrene causes cell loss and dopamine depletion in retinas

isolated from female rats [132], which supports our results and the established association between occupational styrene exposure and impaired vision [133].

Vestibular impairment and balance problems were assessed subjectively via self-reported stumbling while walking, as well as objectively via tests of postural stability and single leg stance. Styrene exposure was associated with increased subjective and objective measures of vestibular impairment for both exposure metrics, apart from the association between blood styrene and measured postural stability. A study of reinforced plastic boat builders also reported impaired postural stability in association with styrene exposure [12], though occupational results are less consistent for vestibular effects.

Sensory impairment was assessed as self-reported numbness/tingling in the extremities, as well as measured vibrotactile threshold. Except for blood styrene and self-reported numbness/tingling, we observed associations between both styrene metrics and both measures of sensory impairment. Occupational studies of subjective sensory impairment, including tingling and numbness, have yielded conflicting results [11, 103, 105]. In studies with objectively measured outcomes, workers exposed to both styrene and toluene exhibited exposure-related changes in somatosensory evoked potentials [109] and painters experienced similar effects [213]. We conducted sensitivity analyses to address other common factors that could contribute to sensory impairment, such as nerve injury and diabetes. Results were unchanged when participants were excluded based on these characteristics, as well as when we adjusted for them.

A dopaminergic mechanism [71] remains the prevailing hypothesis for styrene's mode of neurotoxicity. Several studies suggest that styrene exposure alters dopamine

metabolism and receptors in rodents and humans [126-128]. This mechanism is demonstrated in blood samples of styrene-exposed plastics workers for whom prolactin levels are elevated, as prolactin release is chronically inhibited by dopamine [130], and in male rats where styrene exposure potentiates a dose-dependent decrease in brain dopamine [128].

Taken together, results from Specific Aim 3 offer compelling evidence of styrene's potentially neurotoxic effects at environmental levels (Table 13). Consistent, albeit small, increases in neurologic symptoms among participants with higher ambient styrene exposure are supported by similar relationships of decreases in peripheral neurologic function. While these effect sizes are relatively small, they are of plausible magnitude given that the exposure levels reflect ambient, usual environmental levels across a large geographic region. Associations between blood styrene exposure and both indicators of neurologic effects (symptoms and peripheral neurologic function) were less consistent, but we did observe some suggestive effects for both sets of outcomes. Blood exposure analyses had smaller sample sizes, and were potentially underpowered to detect statistically significant differences of the magnitude we observed. While these findings are based on cross-sectional analyses, and we therefore cannot establish temporality or assess causality, they do provide suggestive evidence of styrene-associated neurologic effects at environmental exposure levels.



## **B. Strengths and limitations**

### **Strengths**

The GuLF STUDY is a large, well characterized cohort with rich exposure information and near complete residential geocoding. Although the present study is not focused on the *DWH* oil spill, this cohort presents a unique opportunity to investigate a novel exposure scenario in an understudied, diverse population.

Blood styrene is a validated biomarker specific to styrene exposure [25]. It has been used extensively in occupational research [6], as well as in general population monitoring [1]. With approximately 1,000 independent measurements and 77% detection, analyses predicting blood styrene measurements were sufficiently well-powered. Moreover, the laboratory provided all measured styrene values, including those below the limit of detection. We were able to use these values as the best estimates of the true styrene level, which is generally preferable to other approaches [180]. The distribution of observed levels supported evaluation of exposure-response relationships and allowed stratification by smoking status, an important predictor of styrene exposure in the general population. Furthermore, the blood styrene exposure range in this population consisted of somewhat elevated blood styrene levels with a broad range of exposures and high rate of detection. This distribution is ideally suited to a predictive analysis that assesses elevated exposure, but is relevant to usual environmental levels.

Although each person provided only one specimen at a single time point, detailed questionnaire data were collected about relevant activities and determinants of VOC exposure occurring at the time of blood collection. Exposure questions were framed with

respect to the 24 hours and 7 days prior to blood collection, as well as over the longer term.

Routine monitoring of ambient styrene across the U.S. does not provide sufficient temporal or spatial coverage to support kriging or other exposure interpolation methods [143, 145, 155] for prediction in Specific Aims 1 and 2. As a result, most GuLF STUDY participants live very far from monitors, negating any meaningful contribution of observed levels. Instead, we integrated two complementary data sources to quantify ambient styrene exposure. NATA generally performs well in comparison to monitored data, incorporates meteorology, uses sophisticated dispersion modeling, and includes all source types, but is limited by spatial resolution based on census tracts. Proximity measures for point sources, while limited in scope of underlying source possibilities, provide enhanced spatial resolution because they are based on geocodes. These measures implement finer dispersion ranges, and facilitate examination of the hypothesized importance of point sources. This method, combining NATA and NEI, has been used to identify HAP hot spots in urban and suburban communities [220].

In Specific Aim 3, we assessed effects associated with long-term ambient styrene and acute internal dose of styrene. Modeling exposure using both proxy and personal metrics helps address possible confounding bias introduced by the biomarker, and potential measurement error of NATA ambient styrene estimates [200]. NATA estimates have been applied as measures of human air pollution exposure in epidemiologic studies of cancer [201-203], asthma [204], birth defects [205], autism spectrum disorder [122, 123, 206], and neurodegenerative diseases [124]. NATA data remain the only valid, spatially-referenced estimation of usual exposure levels with

sufficient geographic coverage for the Gulf state region. Long-term ambient styrene trends indicate that year-to-year regional variation in concentration is not substantial, suggesting that annual average estimates are appropriate assessments of exposure [20]. Stable measured blood styrene levels in independent U.S. population samples, measured cross-sectionally, over a 20-year period corroborate these findings [25, 60, 66]. Blood styrene is a validated biomarker specific to styrene exposure [25] and it has been used extensively in occupational research [6], as well as in general population monitoring [1].

Although self-reported symptoms have been criticized for their subjective nature, they provide a highly sensitive measure of neurologic impairment equipped to detect subclinical, and perhaps early, indications of styrene-induced neurotoxicity [199]. The blood styrene levels observed in the GuLF STUDY are certainly elevated, but they are more similar to general population levels than to occupational levels. As such, these exposures are not expected to elicit severe, pronounced neurologic effects [148], so sensitive neurologic symptoms are well-suited to capture the potentially subtle, widespread neurotoxic effects for this exposure range.

To address potential concern about symptom reporting subjectivity, we additionally employed objective measures of peripheral neurologic function ascertained in a clinical research setting using validated tests [216]. This additional assessment of neurologic impairment allowed us to investigate PNS effects, whereas the occupational literature has focused on CNS effects. Our approach using both subjective and objective outcome assessments also allowed us to evaluate patterns between the

subjective and objective measures, lending potential insight into the validity of symptom reporting in relation to environmental exposures.

## **Limitations**

Although we had a large sample size for a biomarker study, we obtained only a single blood measurement from each individual. Repeated biomarker measurements may provide a more reliable estimate of usual exposure than the single blood specimen obtained in our study, particularly because of the rapid elimination of styrene from the body. Given the half-life of blood styrene, we are limited in our interpretation of the blood level relative to neurologic outcomes. It should be noted that symptom ascertainment predated the blood draw, whereas peripheral neurologic function was tested after blood collection. Therefore, timing-related exposure misclassification may have led to underestimation of effects, unless all measured exposures truly reflect usual daily exposure. The observed similarity of findings across exposure metrics and outcomes, however, suggests that temporality issues did not entirely obscure associations.

Lacking detailed information on styrene-specific occupational exposure opportunities, we assessed reported industry, occupation, and activity information for participant's recent and longest-held jobs. Occupational data were reported at enrollment and potentially not reflective of the relevant 24-hour exposure window preceding the blood draw. Despite this limitation, it is unlikely that occupational styrene exposure is influencing blood styrene levels in this study population because styrene-related occupations tend to be highly specific and reported rarely among CBS participants. We searched through self-reported industry/occupation information for any

potentially styrene-related work experience, with the intention of adjusting for work or excluding these individuals from analyses. Ultimately, occupational styrene exposure opportunities were so rare in our study population (0.1%), that we concluded that occupational exposures were not influencing results.

Reporting to the NEI database is voluntary and designed for regulatory purposes. As such, the quality of the data and availability of information for our intended investigation may not be ideal. These data are limited to annual aggregate values, and lack any temporal specificity. Similarly, the NATA estimates are annual averages derived from voluntarily reported inputs, the protocols for which vary between states. Although imperfect, in the absence of sufficient monitoring data, these data sources provide the best available outdoor exposure information for a study of our scope and purpose. Although the assumptions inherent to an annual average estimate of air pollution potentially limit interpretation for acute exposure scenarios, NATA data are a valid estimation of usual exposure levels experienced in the Gulf region.

Due to the cross-sectional nature of our study, we could not evaluate the relevant timing of exposure and outcome in Specific Aim 3. Associations examining neurologic symptoms are conceptually cross-sectional, though exposure assessment was completed after outcome ascertainment in sub-analyses examining associations between blood styrene levels and neurologic symptoms among CBS participants. Blood measurements were obtained within three months of enrollment for 47% of participants, and approximately 70% of all home visits were completed within six months of enrollment. Scheduling was not related to oil spill cleanup or other exposures, so the

interval duration between outcome and exposure assessment is expected to be randomly distributed.

Because blood styrene measurements reflect recent exposure, assessing exposure after outcome ascertainment is a potential limitation. There is no biological basis to assume that impaired neurologic function would affect subsequent blood burden of styrene, so reverse causality seems unlikely. However, if the measured blood styrene reflects transient exposures encountered before or after outcome ascertainment, exposure misclassification would potentially bias associations between blood styrene and neurologic outcomes. We conducted sensitivity analyses restricted to participants who completed the home visit within three and six months of enrollment, and found that results were unchanged. This suggests that the temporal lag did not introduce exposure misclassification to bias associations.

### **C. Public health significance**

Given that styrene use and generation are widespread, and inhalation of airborne styrene is the principal route of exposure, it is a critical public health priority to evaluate whether adverse health effects may result from chronic ambient exposure at levels relevant to the general population. Our research lent limited insight into the underlying sources responsible for styrene exposure in this population and region. This was the first study to examine the neurologic effects of styrene exposure of this magnitude. The combined findings from our analyses suggest that styrene is an important exposure for human health effects, and that more research is warranted to determine policy implications.

Our investigation into ambient styrene in relation to neurologic symptoms suggests that styrene's neurotoxic properties may be apparent even at exposure levels relevant to the general population. The relationship between blood styrene exposure and neurologic symptoms was equivocal, but suggestive of an association for certain endpoints. People exhibiting the symptoms evaluated in our study may be at risk for more severe neurologic impairments or disease, as these outcomes may be early markers of more severe manifestations. Furthermore, the large fiber function abnormality we detected via objective neurologic testing is a possible early indicator of peripheral neurological disease.

Future research is needed to determine whether styrene-induced neurotoxicity derived from environmental exposures persists over time, and how it may relate to neurological disease. In the presence of a ubiquitous exposure like air pollution, even subtle neurologic effects can have significant impacts on population-level health effects. Implementing policies and styrene exposure mitigation efforts could lead to widespread, incremental improvements in neurologic health at the population level.

#### **D. Directions for future research**

While occupational styrene levels have been characterized fairly extensively, environmental exposure levels and their underlying sources remain less clear. Most exposure assessment in the general population has been cross-sectional and monitoring is limited. To improve elucidation of determinants of styrene exposure, as well as typical environmental levels, valuable future research could assess exposure using repeated measures in varying media.

Based on the apparently bimodal distribution of blood styrene levels in the CBS cohort, we could potentially improve prediction of blood styrene exposure using finite mixtures modeling [221]. This approach offers a parametric alternative to an unknown distribution like the one we observed, potentially accounting for unobserved heterogeneity in our data [222]. Ideally, this would allow us to predict the likelihood of membership in different ranges of the exposure distribution, potentially revealing previously unrecognized determinants of elevated blood styrene levels.

GuLF STUDY data collection and follow-up are ongoing, creating an opportunity to evaluate associations between styrene exposure and neurologic effects longitudinally. Our current research is limited to cross-sectional data, and establishing temporality through a prospective analytic design would strengthen results, and the interpretations of relationships between styrene exposure and neurologic effects.

Because humans are commonly exposed to multiple correlated air pollutants simultaneously [223], it is important to build on observed associations with individual chemical exposures in isolation by evaluating exposure mixtures. Styrene is highly correlated with BTEX in air and blood, as these VOCs share many common environmental sources [1, 60, 84]. BTEX are also neuroactive [78-80, 224, 225]. We can extend the present analyses by considering BTEX and styrene exposure mixtures in air and blood using penalized regression or other statistical methods [226, 227], including weighted quantile sums, lasso, ridge, elastic net, or Bayesian kernel machine regression [228]. Implementing a mixtures-focused approach would potentially yield important benefits for the public health impact of this research [229], as well as further insight into environmental styrene neurotoxicity.



NATA data are currently the best available proxy measures for ambient styrene exposure across the Gulf region, though a lack of temporal specificity limits their utility. More sophisticated air quality modeling that incorporates temporal variability would improve environmental exposure assessment for both analyses: predicting blood styrene levels, as well as investigating the relationship between styrene exposure and neurologic effects, especially if some portion of the effects is transient. Further, enhanced or targeted monitoring of ambient styrene levels would potentially improve the validity of modeled environmental styrene concentrations [162, 230].

Beyond the scope of this cohort, objective evaluation of neurologic impairment in occupational settings has largely been restricted to CNS effects, commonly measured through neurobehavioral and psychological testing. Applying our comprehensive battery of peripheral neurologic function testing to a new or existing occupational cohort would contribute meaningfully to the understanding of styrene-related PNS impairment, a relationship that remains unclear.

Table 13. Summary of findings for ambient and blood styrene exposure in relation to neurologic effects.

Peripheral Neurologic function	Styrene exposure type		Neurologic symptom	Styrene exposure type	
	Ambient	Blood		Ambient	Blood
Contrast Sensitivity	Association (Q4)	Association (P90)	Blurred vision	Association (Q3, Q4)	<i>Null</i>
Vibrotactile Threshold	Association (Q4)	Association (P90)	Tingling/Numbness	Association (Q4)	<i>Null</i>
Single Leg Stance	Association (All)	Association (P90)	Stumbling	Association (Q4)	Association (Median)
Postural Stability	Association (All)	<i>Null</i>			

Positive denotes evidence of an association between styrene exposure and impaired neurologic function or increased risk of neurologic symptoms. *Null* denotes lack of evidence for an association between styrene exposure and neurologic effects.

Q3, statistically significant for third quartile.

Q4, statistically significant for fourth quartile.

P90, statistically significant for exposure above the 90<sup>th</sup> percentile.

All, statistically significant for all exposure quartiles.

Median, statistically significant for exposure above the median value.

**APPENDIX 1. DISTRIBUTION OF EPA MONITORS AND GULF STUDY PARTICIPANTS**

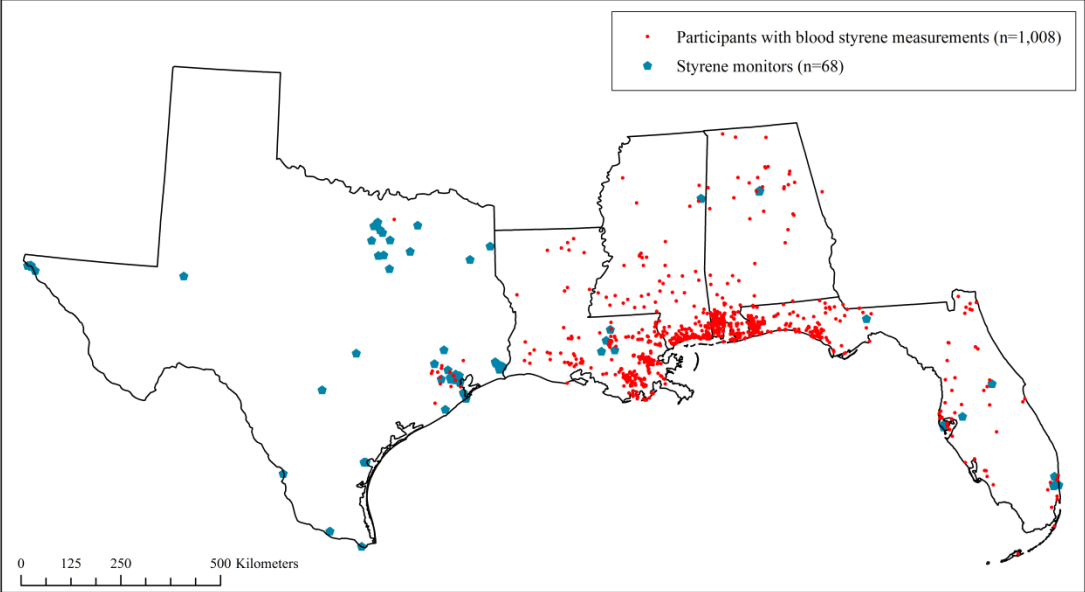
Table A1. Distribution of Gulf STUDY biomonitoring sub-study participants' (N=1,055) distance to nearest styrene monitor

	2011 monitors (N=44)		2013 monitors (N=68)	
	Miles	Kilometers	Miles	Kilometers
Mean (SD)	114.4 (56.5)	184.2 (91.0)	134.5 (69.8)	216.5 (112.3)
Minimum	1.7	2.7	0.3	0.5
25 <sup>th</sup> percentile	62.6	100.8	68.0	109.5
Median	119.5	192.4	145.9	234.8
75 <sup>th</sup> percentile	170.1	273.7	186.6	300.3
Maximum	213.3	343.2	259.9	418.2

Table A2. Number of Gulf STUDY biomonitoring sub-study participants (N=1,055) with  $\geq 1$  styrene monitor within a given radius (*buffers drawn around participants*)

Radius (miles)	All 2011 monitors (N=44)	NATA 2011 paired monitors (N=15)	All 2013 monitors (N=68)
	No. people	No. people	No. people
1	0	0	2
2	1	1	3
5	9	6	9
10	31	22	30
20	62	44	56
50	171	142	154

Figure A1. Blood measurements and monitors in the Gulf region



## **APPENDIX 2. EVALUATION OF NATIONAL AIR TOXICS ASSESSMENT BY STATE**

Owing to concerns about inter-state variability in NATA data quality and reporting, we examined agreement between NATA estimates and monitored concentrations in corresponding census tracts by state (Table A1). Treating the annual average of observed values at monitors as the true estimate of exposure at that location, NATA performance is best in Louisiana and Texas, followed by Mississippi and Florida. Alabama emerged as potentially problematic based on the large disparity with monitored data. Estimated concentrations in Alabama are furthest from monitored concentrations, with a marked difference from the other Gulf states. This analysis is very limited by the sparsity of monitoring coverage, but given the general lack of data availability, we felt that any additional information contributed to our evaluation.

Based on the patterns observed in Table A1, we examined effects of differential reporting to NATA by state on associations between ambient and blood styrene levels. Given insufficient sample sizes in each individual state to support state-specific analyses, we instead conducted four parallel analyses eliminating one state each time (Figure A1). Results were fairly consistent across analyses, apart from the removal of Alabama. When participants from Alabama were excluded, we observed an association between nonpoint ambient exposure and elevated blood styrene.

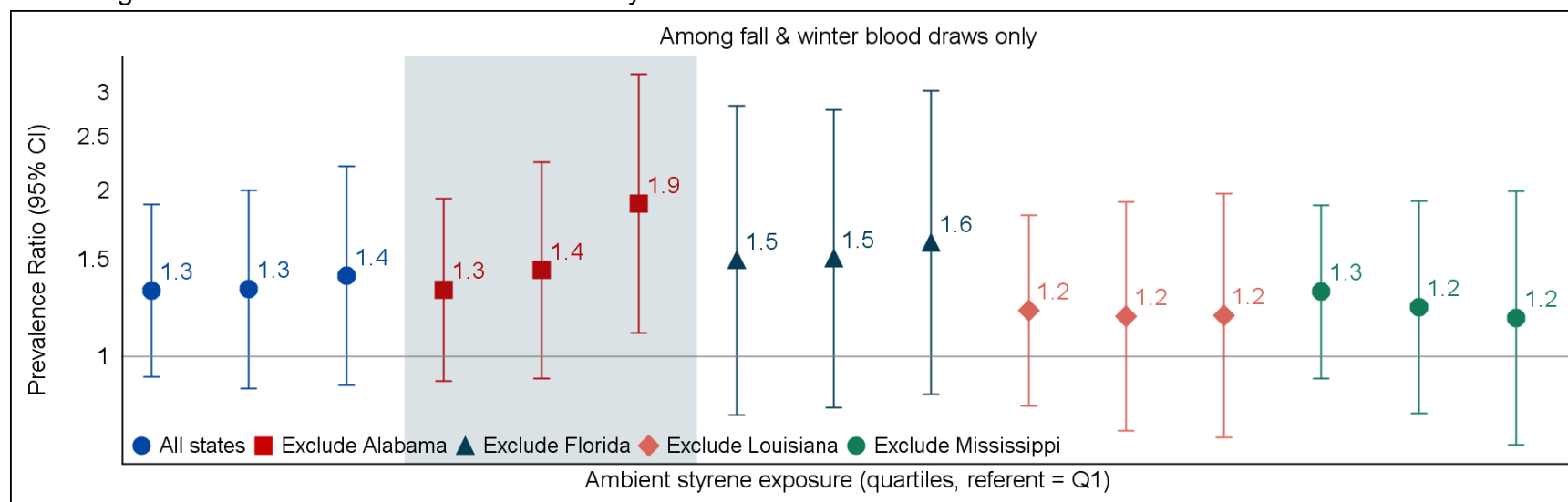
We hypothesized that NATA data may represent different underlying information in Alabama, as compared with the other Gulf states. We used this information to select a subpopulation in which we had higher confidence in NATA data, ultimately excluding participants from Alabama for sensitivity analyses that were focused on NATA estimates of exposure.

Table A3. Comparing NATA 2011 estimated annual concentrations and Ambient Monitoring Archive observed annual concentrations by Gulf state.

State	Number of monitors	Average difference ( $\mu\text{g}/\text{m}^3$ ) (Monitors – NATA)	Average Ratio (Monitors / NATA)
Alabama	3	0.67	27.3
Florida	9	0.23	11.9
Louisiana	4	0.07	1.7
Mississippi*	2	0.08	4.4
Texas	19	0.07	1.3

\* No monitoring data available in 2011; the only two active monitors from 2010 were used instead.

Figure A2. Association between ambient styrene quartiles and elevated blood styrene (> NHANES 95<sup>th</sup> percentile), excluding one state at a time to assess variability in NATA.



Adjusted for 2,5-dimethylfuran (log), state, season, and time in vehicles ( $\geq 3$  hours).

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