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Blood BTEX levels and neurologic symptoms in Gulf states residents

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Abstract

Background: The chemicals benzene, toluene, ethylbenzene, and xylenes (BTEX) are neuroactive. Exposures often co-occur because they share common sources. We examined neurologic effects of environmental BTEX exposure among U.S. Gulf coast residents taking into account concomitant exposures.

Methods: We measured blood concentrations of BTEX in 690 Gulf state residents. Neurologic symptoms were ascertained via telephone interview. We used log-binomial regression to estimate associations between blood BTEX levels and self-reported neurologic symptoms independently for the presence of any neurologic, central (CNS), or peripheral nervous system (PNS) symptoms. We estimated associations in single chemical models mutually adjusted for co-occurring BTEX and used weighted quantile sum regression to model associations between the combined BTEX mixture and neurologic symptoms.

Results: Half (49%) of participants reported at least one neurologic symptom. Each BTEX chemical was associated with increased CNS and PNS symptoms in single-chemical models comparing the highest to lowest quartile of exposure. After adjusting for coexposures, benzene was associated with CNS symptoms among all participants (PR=2.13, 95% CI: 1.27, 3.57) and

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among nonsmokers (PR=2.30, 95% CI: 1.35, 3.91). After adjusting for coexposures, associations with toluene were apparent only for reporting multiple PNS symptoms (PR=2.00, 95% CI: 0.96, 4.16). In mixture analyses, a one-quartile increase in BTEX exposure was associated with neurologic symptoms (OR=1.47, 95% CI: 1.11, 1.98). The weighted quantile sum index weighted benzene most heavily, which was consistent with single chemical analyses.

Conclusions: Increasing blood benzene concentration was associated with increased prevalence of CNS symptoms. In this sample, BTEX-associated neurologic effects are likely driven by exposure to benzene and, to a lesser extent, toluene.

Keywords

biomarker; neurologic; benzene; mixture; volatile organic compound

INTRODUCTION

The volatile organic compounds (VOCs) benzene, toluene, ethylbenzene, and xylenes, collectively known as BTEX, are a ubiquitous mixture in the environment. These chemically similar compounds are the primary constituents in crude oil and are commonly found in automobile exhaust, tobacco smoke, and industrial emissions from petroleum and coal combustion [1].

The general population is exposed to BTEX predominantly from inhalation of airborne emissions and cigarette smoke, though exposure from ingestion of contaminated water and food is also possible [2]. Oral and dermal exposures are generally more common in occupational settings [3–6]. Increased levels of ambient BTEX occur in certain industrial areas, high traffic locations, and near gas stations and hazardous waste sites [7, 8]. BTEX levels are typically higher in indoor than outdoor air, a result of off-gassing from building materials and consumer products, as well as lower air circulation rates [9, 10].

BTEX, all classified as hazardous air pollutants, can impair liver, kidney, respiratory, and immune function, and cause DNA damage and hematologic effects [3–6, 11–14]. Research has demonstrated the adverse effects of benzene, a known carcinogen [15], but disentangling effects of BTEX coexposures is a common limitation of health effects studies. The nervous system is considered a critical target organ for the four chemicals [16]. BTEX-related neurotoxicity has been examined in experimental studies in animals and humans [3–6], and occupational settings [17–20], with inconsistent results. Associations are compelling at higher exposure levels, but epidemiologic research examining the relationship between environmental BTEX exposure and neurotoxicity is lacking.

In this study, we evaluate associations between blood BTEX levels and neurologic symptoms in Gulf coast residents who were transiently exposed to BTEX during the *Deepwater Horizon* (*DWH*) oil spill and/or response. The measured blood BTEX levels here are not related to oil spill hydrocarbon exposures because BTEX have short half-lives reflecting recent exposure (past 24 hours) [21, 22], and these blood specimens were obtained years after the oil spill. Indeed, despite living in a region with prolific petrochemical and

industrial operations, blood BTEX levels in this population are comparable to population levels measured in the National Health and Nutrition Examination Survey (NHANES) [23].

METHODS

Study Design and Participants

We used data from the Gulf Long-term Follow-up Study (GuLF Study), a prospective cohort of individuals (ages 21 and older) who participated in oil spill response activities and others who received safety training, but were not hired, following the 2010 *Deepwater Horizon* (*DWH*) disaster. Participants enrolled between 2011 and 2013. A detailed description of this study is available elsewhere [24, 25]. Approximately 2–3 years after the disaster (May 2012-July 2013), a convenience sample of GuLF Study participants living in the Gulf region (N=1,055) were enrolled in the Chemical Biomonitoring Study (CBS), as described elsewhere [26]. CBS participants provided a blood sample used to measure VOCs as part of a home visit for participants residing in this region. A portion of ethylbenzene samples were excluded due to analytical measurement interference, thereby limiting the available sample with complete analyte quantification of the BTEX mixture. Due to our interest in exposure to the BTEX chemical mixture, we restricted the present analyses to participants who had complete quantification of all BTEX analytes (n=724). After excluding 24 participants missing neurologic symptom information and ten individuals missing covariate information, we arrived at a final analytic sample of 690.

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

Blood biomarker measurement

Blood specimens for biomarker measurement were obtained during the CBS home visit, between May 2012 and July 2013. Blood collection tubes containing potassium oxalate and sodium fluoride anticoagulant were used to collect 10 mL of blood for VOC measurement. Tubes and stoppers were pre-treated by the Centers for Disease Control and Prevention (CDC) laboratory to remove VOC residues to minimize pre-collection contamination [27, 28]. 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. VOCs were analyzed using equilibrium headspace solid-phase micro-extraction with benchtop gas chromatography/mass spectrometry following standard CDC procedures [29, 30]. In statistical analyses, ortho-and meta−/−para-xylene concentrations were combined for an overall measure of total isomeric xylenes. Cotinine, a biomarker of exposure to tobacco smoke, was measured in serum that was stored in gas-phase nitrogen until analysis [25]. Cotinine analysis was performed using liquid chromatography/mass spectrometry [31]. The laboratories provided actual measured values below the limit of detection (LOD; benzene, 0.024 ng/mL; toluene, 0.025 ng/mL; ethylbenzene, 0.024 ng/mL; ortho-xylene, 0.024 ng/mL; meta-/para-xylene, 0.034 ng/mL; cotinine, 0.015 ng/mL).

Neurologic symptoms

Health information, including neurologic symptoms, was collected via Computer Assisted Telephone Interview (CATI) during the enrollment interview, which occurred a median of 98 days prior to the CBS exposure ascertainment. Participants were asked to report how often they experienced dizziness, nausea, headaches, sweating, palpitations, tingling, numbness, blurred vision, stumbling while walking, and fatigue during the preceding 30 days. Frequency of 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.

Based on results of a principal components analysis of 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.

Covariate information

Covariates were selected using a directed acyclic graph [32]. Covariate information was obtained during the enrollment interview. We adjusted all models for sex (male, female), age (<30 years, 30–45, >45), season of enrollment (winter, spring, summer, fall), race (white, black, other), employment status (currently working, not currently working), current use of alcohol consumption (any self-reported alcoholic drinks in the last year, no drinks in the last year), smoking (self-reported current smokers, nonsmokers), educational attainment (completed less than high school diploma or general equivalency degree (GED), completed high school diploma/GED, completed some college, college graduate), annual income (≤ $20,000, $20,001–50,000, >$50,000), interval between enrollment and blood draw (days), diabetes (self-report of doctor diagnosis), and estimated maximum total hydrocarbon (THC) exposure during *DWH* spill cleanup (non-worker, ≤0.29 ppm, 0.3–0.9 ppm, 1.0–2.99 ppm, ≥3.0 ppm). Maximum THC exposure experienced during the oil spill response and cleanup was estimated by GuLF Study industrial hygienists linking air measurements taken during the spill from passive dosimeters with each participant's interview responses about oil spill work experiences [33]. We imputed the jointly defined sex-race-state-specific median income from the analytic population as a proxy for income when it was missing (n=37).

Single chemical analysis

We separately examined associations between individual BTEX levels and the presence of any neurologic symptom, any CNS symptom, more than one CNS symptom, any PNS symptom, and more than one PNS symptom. As a secondary analysis, we estimated associations between BTEX levels and individual neurologic symptoms. Due to model convergence problems related to lower prevalence of individual symptoms, we modeled these associations using modified Poisson regression. We used all measured blood BTEX values, including the measured values below the LOD [34], to rank levels of each BTEX chemical into quartiles for modeling. The lowest quartile for a given chemical was the referent exposure category in all analyses. We used multivariate log-binomial regression to estimate prevalence ratios and corresponding 95% confidence intervals (PR, 95% CI) for the cross-sectional associations between blood BTEX levels and neurologic symptoms. We

evaluated exposure-response relationships using Wald tests of linear trend and reported corresponding p-values. For each of the BTEX chemicals, we used two covariate adjustment sets to estimate associations: first, we modeled only the covariates identified by the directed acyclic graph ($PR_{covariates}$); second, for coexposure-adjusted analyses, we added all BTEX compounds (quartiles) to covariate-adjusted models ($PR_{coexposures}$). Given that smoking is a principal source of BTEX exposure in this population [23], we also examined coexposure adjusted associations among nonsmokers only ($PR_{nonsmokers}$, n=466).

To further examine the influence of exposure to tobacco smoke in the overall sample, we conducted separate analyses additionally adjusting for self-reported exposure to environmental tobacco smoke (ETS, defined as living in a home with a regular smoker at enrollment), continuous serum cotinine values, and sensitive cotinine thresholds to reflect active smoking (0 ng/mL vs >0–10 ng/mL vs > 10 ng/mL) and ETS (dichotomized at LOD, 0.015 ng/mL) exposure [35]. We conducted the following sensitivity analyses: we stratified on the median interval between date of symptom reporting and the blood draw (98 days), we stratified on the median interval between the last day of oil spill cleanup work and the blood draw among *DWH* response and cleanup workers (839 days, n=585), we evaluated heterogeneity by race using interaction terms between exposure and race (white vs nonwhite), and we evaluated the impact of excluding potentially influential observations.

Mixture analysis

Because BTEX are highly correlated and share common sources, we used weighted quantile sum (WQS) regression to estimate associations between exposure to the combined BTEX mixture and neurologic symptoms [36]. This approach generates a weighted linear index of the body burden of total BTEX exposure and estimates odds ratios (OR, 95% CI) associated with ranked exposure to the mixture. We used this approach because it is a validated, statistically efficient way to model the likely scenario in which participants are exposed concomitantly to BTEX [37, 38].

To assess suitability of the WQS approach for mixture analysis, we evaluated potential interactions among BTEX in single chemical models adjusted for covariates and coexposures. Interactions were modeled as product terms between the parameter for the main BTEX exposure of interest (quartiles) and the modifying coexposure (above or below the median).

To facilitate comparability to results from single chemical models, we modeled the combined association between a one-quartile change in the BTEX mixture and neurologic symptoms. Because neurologic symptoms were relatively common in this population (>10%), odds ratios do not directly approximate risk ratios. Therefore, we additionally included transformed effect estimates (square root of the odds ratio) for more intuitive comparison to the prevalence ratios estimated in single chemical analyses [39].

The dataset was randomly divided into a training and validation dataset (50% training, 50% validation) and bootstrap sampling (n=100) of the training dataset was used to estimate weights quantifying the relative importance of each component chemical in the exposure mixture. These weights, which are constrained from zero to one and sum to one, can be

interpreted as the relative importance of each chemical component driving associations. For BTEX, a mixture with four components, weights of 0.25 indicate balanced contributions by each chemical component. The WQS approach performs well in the presence of highly correlated exposures, demonstrating high sensitivity and specificity for selecting important chemical components [37].

We initially considered including n-hexane in exposure mixture analyses because it is a known neurotoxin and constituent of crude oil [40]. However, we ultimately omitted it due to low detection (< 5%) and low correlation with BTEX ($r < 0.10$), concluding that we lacked sufficient power to detect associations with n-hexane.

We conducted all statistical analyses apart from WQS in SAS 9.4 (Cary, NC, USA). We used the R package gWQS for WQS mixture analyses [41].

RESULTS

Half (49%) of participants reported at least one neurologic symptom and nearly one-third reported any CNS or any PNS symptom (32% and 31%, respectively). Participants who experienced symptoms were more likely to be nonwhite and to report smoking, diabetes, unemployment, and lower education and income than their counterparts with no neurologic symptoms (Table 1). Median benzene and toluene levels were higher among those who reported symptoms, but median ethylbenzene and xylenes appeared similar by neurologic outcome. Overall, Spearman correlations among BTEX ranged from 0.75 to 0.92 (Table 2). Blood levels were highest for toluene and lowest for ethylbenzene. The distribution of blood BTEX did not differ by inclusion or exclusion from the analytic sample (Supplemental Table 1). Excluded participants reported lower rates of PNS symptoms but were similar to the analytic sample with respect to other outcome clusters (Supplemental Table 2).

In covariate-adjusted models without coexposures, increasing blood benzene was associated with all outcomes except multiple PNS symptoms (Figure 1, Supplemental Table 3). In all models (i.e., without coexposures, with coexposures, and among nonsmokers), we observed significant linear trends for increasing benzene and CNS symptoms (p for trend ≤ 0.02). We also observed consistent associations for the highest quartile of benzene and any CNS ($PR_{covariates}$=2.05, 95% CI: 1.45–2.89; $PR_{coexposures}$=2.13, 95% CI: 1.27–3.57; $PR_{nonsmokers}$=2.30, 95% CI: 1.35–3.91) and multiple CNS symptoms ($PR_{covariates}$=2.56, 95% CI: 1.44–4.57; $PR_{coexposures}$=3.64, 95% CI: 1.60–8.25; $PR_{nonsmokers}$=2.34, 95% CI: 1.13–4.84). While PRs for associations between the highest quartile of benzene exposure and all symptoms were greater than 1.0, individual symptom analyses lacked precision (Supplemental Table 4).

The highest quartile of blood toluene was significantly associated with all outcomes in covariate-adjusted models, but associations were generally not evident in models mutually adjusted for coexposures (Figure 2). We did observe suggestive associations between the fourth quartile of toluene and multiple PNS symptoms in all models ($PR_{covariates}$=1.54, 95% CI: 0.98–2.41; PRcoexposures=2.00, 95% CI: 0.96–4.16; $PR_{nonsmokers}$=3.11, 95% CI: 1.13–8.52), with the strongest association among nonsmokers. Overall, we did not detect a

relationship between toluene and CNS symptoms. We did, however, observe an unexpected protective association for toluene and multiple CNS symptoms among nonsmokers.

The highest quartile of ethylbenzene was associated with any neurologic ($PR_{covariate}$=1.37, 95% CI: 1.09–1.71), any CNS ($PR_{covariate}$=1.46, 95% CI: 1.07–1.99), and any PNS symptom ($PR_{covariate}$=1.39, 95% CI: 1.03–1.89) in covariate-adjusted models, however associations were no longer apparent when coexposures were included or when restricted to nonsmokers (Supplemental Figure 1).

Xylenes were associated with most outcomes in covariate-adjusted models, but most of these associations appear to be confounded by coexposures (Supplemental Figure 2). In models adjusting for coexposures and restricting to nonsmokers, we did, however, observed large, albeit imprecise associations for multiple CNS symptoms with the second ($PR_{nonsmoker}$=2.44, 95% CI: 1.40–4.22), third ($PR_{nonsmoker}$=7.15, 95% CI: 2.99–17.07), and fourth quartiles of exposure ($PR_{nonsmoker}$=4.11, 95% CI: 1.36–12.45) and evidence of significant exposure-response trend (p for trend=0.005).

In general, results did not change appreciably when we adjusted for self-reported ETS or serum cotinine levels (either continuous or thresholds) (Supplemental Tables 5–6). Analyses stratified by median interval since enrollment or cleanup were underpowered due to small sample size, so estimates appeared unstable (data not shown) and we could not estimate associations with less common outcomes (multiple CNS, multiple PNS). Nevertheless, we did not detect evidence of confounding by interval. We were similarly limited in our ability to assess heterogeneity by race because stratified analyses yielded imprecise results due to smaller sample size. Overall, interaction terms did not suggest effect modification by race. In descriptive analyses, we did observe that nonwhite participants were more likely to experience symptoms (nonwhite, 57%; white, 42%), had higher median benzene (nonwhite, 0.07 ng/mL; white, 0.03 ng/mL) and toluene levels (nonwhite, 0.23 ng/mL; white, 0.16 ng/mL), and reported lower socioeconomic status than white participants, though we did not observe differences in smoking patterns by race (Supplemental Table 7). The analytic sample included two participants with blood toluene levels higher than five ng/mL. Even though these levels are biologically plausible, because they were notably higher than the remaining distribution of blood toluene, we evaluated their influence on results by removing them from all analyses. Results changed minimally when the potentially influential observations were removed (data not shown).

We didn’t observe evidence of pairwise interactions among BTEX, though some models failed to converge (data not shown). Suggestive, albeit inconsistent, evidence of interaction between benzene and toluene was likely the result of sample size limitations (cell sizes < 5) arising from the high correlations (0.75–0.92) among BTEX in these data. We lacked precision to compare stratified estimates where we did see potential heterogeneity because estimates were based on very small cell sizes.

In mixture analyses, a quartile increase in the weighted quantile sum index of the BTEX mixture was associated with a statistically significant 47% increase in odds of reporting any neurologic symptom (OR=1.47, 9% CI: 1.11–1.98). We observed a significant association

with any CNS symptom (OR=1.40, 95% CI: 1.05–1.89) and a suggestive association with any PNS symptoms (OR=1.25, 95% CI: 0.93–1.69) (Table 3). Transformed odds ratios for associations with a quartile increase in the BTEX mixture ranged from 1.06 (multiple PNS symptoms) to 1.21 (any neurologic symptoms). Benzene was weighted most heavily for all symptoms (range of weights=0.55–0.87), suggesting that, in the BTEX mixture, benzene exerts the strongest association with these outcomes. Notably, toluene appeared to be a relevant agent in the association with multiple PNS symptoms (weight=0.36).

DISCUSSION

In this study examining associations between environmental BTEX and neurologic symptoms, we observed that increasing blood benzene levels were consistently associated with central nervous system effects. We observed monotonic exposure-response relationships between benzene and CNS symptoms, as well as between toluene and PNS symptoms, even after accounting for coexposures in the overall population and among nonsmokers. These findings were underscored by WQS analyses, in which the BTEX mixture was associated with increased CNS and PNS symptoms. Mixture analyses identified benzene as the primary agent driving associations, with some indication of an additional contribution by toluene, particularly in relation to PNS symptoms. This is, to our knowledge, the first biomarker-based study to evaluate the relationship between environmental BTEX exposure and neurologic symptoms.

Associations with blood benzene levels were strongest with multiple CNS symptoms when adjusting for coexposures. Previous epidemiologic studies in occupational settings have emphasized toluene as an important neurotoxicant [6]. A toluene effect does appear in our data, but our results suggest that benzene is more important and may be neuroactive at low exposure levels. Our results from mutually BTEX-adjusted and WQS models are consistent with this, and WQS weightings indicate that benzene is the most important ‘bad actor’ in the mixture. This finding is novel regarding neurologic endpoints, but these blood benzene levels were also associated with modest adverse hematologic effects in this population [42]. Further, low-level occupational exposure to benzene (<1 part per million) was associated with hematotoxicity in a study of shoe workers [43]. A review of environmental exposure to BTEX and health effects concluded that each component in this exposure mixture may demonstrate endocrine disrupting properties even at reference exposure levels [2]. Our study, together with these findings, lends plausibility to health effects from environmental levels of benzene exposure and, perhaps, the other BTEX components.

In analyses of blood toluene, we observed consistent associations and monotonic exposure-response relationships for participants reporting multiple PNS symptoms. The lack of association between toluene and other symptom clusters may be due to the relatively high levels of toluene compared to the other chemicals in the mixture. Because the referent exposure group in toluene analyses had universally detectable levels, the lowest quartile included participants with higher exposures than for other chemical analyses. As such, associations may have only been apparent among those with a stronger neurologic response, indicated by more co-occurring symptoms. The inverse association between toluene and multiple CNS symptoms among nonsmokers is not consistent with other outcomes (any

neuro, any CNS), and may be underpowered, as associations with this outcome are potentially unstable as sample size diminishes. We are unaware of any mechanism supporting the plausibility of toluene's neuroprotective effects on the CNS. This association may be an artifact of selection in this nonsmoking subgroup. Toluene's neurotoxic effects on the CNS and PNS have been demonstrated experimentally in animals and in occupational settings [6, 17].

Consistent with our findings, environmental ozone exposure has been associated with CNS symptoms, including headache and fatigue [44]. Ground-level ozone, a secondary pollutant formed by photochemical reactions of BTEX with nitrogen oxides, may activate and increase levels of the pro-inflammatory cytokines interleukin one beta (IL-1β) and tumor necrosis factor alpha (TNF-α) [45]. Increases in IL-1 β and TNF-α can induce oxidative stress in the CNS and alter the blood brain barrier. Although the mechanisms underlying the relationship between BTEX and neurologic function are not fully understood, BTEX can also cross the blood brain barrier and directly affect the CNS [46]. BTEX induce oxidative damage at low occupational levels [12–14], suggesting that the oxidative stress mechanism hypothesized for ozone exposure and neurologic effects may apply to direct BTEX-related neurotoxicity as well.

Given that the weight of evidence for BTEX-associated neurotoxicity emphasizes benzene and toluene, the lack of associations between blood ethylbenzene or xylenes and neurologic symptoms in our study is not entirely surprising. Ethylbenzene's neurotoxicity has been confirmed in experimental animal studies, but associations in humans are limited to populations occupationally exposed to solvent mixtures [5]. Exposure assessment in these studies of solvent mixtures prevents inference about ethylbenzene specifically. Research on xylenes is similarly weakened by limitations in exposure assessment and presence of coexposures [4], though one experimental and one occupational study found slight increases in some CNS symptoms, including nausea and sensation of floating.

Because smoking and ETS account for the majority of environmental benzene exposure, we conducted sensitivity analyses evaluating the influence of smoking on associations. Compared to overall results adjusting for self-reported smoking, results were largely similar when we restricted to nonsmokers, additionally adjusted for ETS exposure, or adjusted for serum cotinine. Consistency of findings in these smoking-related analyses suggests that associations with neurologic symptoms appear to be independent of smoking or any potential smoking-related measurement error. Some associations were slightly strengthened in nonsmokers, indicating that exposure to environmental sources of BTEX may be sufficient risk factors to alter neurologic health in the absence of smoking. That our findings were robust to multiple sensitivity analyses addressing smoking is a considerable strength of this study.

The sample size, while large for a biomarker study, was still small for many subgroup analyses. Based on previously described differences in neurologic symptoms among racial subgroups in this population [47], we intended to evaluate heterogeneity by race in stratified analyses. In general, interpretations did not vary between race-stratified and overall analyses, though incomplete model convergence for some outcomes indicated that the analysis was

underpowered. Because exposure to both elevated chemical exposures and social stressors can have synergistic health effects [48], any differences between groups are likely driven by underlying differences in the distributions of exposures, outcomes, and socioeconomic factors by race. In this population, nonwhite participants experienced higher exposures, lower socioeconomic status, and more neurologic outcomes.

An important strength of this study is that we evaluated neurologic effects of each BTEX chemical exposure separately, mutually adjusted, and as an exposure mixture. We measured internal dose for each component of the mixture using validated biomarkers [49] and used laboratory methods optimized to accurately quantify low-level exposures observed at environmental levels [50]. Previous observational and occupational research has been limited by incomplete, or indirect, exposure assessment, introducing potential confounding by coexposures [51]. By modeling total BTEX exposure in mixture analyses, we employed statistical methods that allowed us to address this confounding, evaluate realistic exposure scenarios, and identify which chemicals were driving associations. We observed that interactions were unlikely in our sample, which is consistent with the ATSDR determination that joint neurotoxic actions among BTEX are likely additive at environmental levels [16]. Given that there is no evidence supporting interactions or departures from additivity among BTEX at environmental levels on the nervous system in the literature [16], we did not detect compelling evidence of interactions, and that WQS performs well in the presence of highly correlated mixtures [37], this is an appropriate, efficient method to model these exposures.

Although we observed significant exposure-response relationships for increasing benzene and toluene in single chemical analyses, we could not evaluate trends in mixture analyses because WQS assumes linearity. We did, however, attempt to make interpretations of mixture analyses comparable to single chemical results by modeling a quartile change in the mixture and providing transformed odds ratios to better approximate prevalence ratios in the presence of common outcomes. Given that effect estimates in mixture analyses were less than two and outcome prevalence was under 50% for all clusters, odds ratios are unlikely to depart materially from prevalence ratios [52], but we provided both measures for ease of interpretation. Indeed, interpretations were similar between odds ratios and transformed estimates.

Limitations of this study, such as its cross-sectional design and reliance on a single blood measurement, prevent us from making any inferences related to causality or timing of exposures and outcomes. Although blood BTEX are valid biomarkers of internal burden widely used in occupational and general population biomonitoring [3–6], they reflect recent exposures only. As such, we introduce potential exposure misclassification by assuming that blood BTEX levels in our study, which were obtained 1–631 days after symptom reporting, are representative of usual exposure, and specifically of the levels present at the time of symptom reporting. The impacts of this misclassification on results are likely minimal, given that we did not observe evidence of heterogeneity in analyses evaluating modification by the duration of the interval between symptom reporting and exposure ascertainment. All models were adjusted for the duration of this interval, though this parameter did not contribute significantly to model fit. Further, previous analyses of these data indicate that smoking, sex, age, socioeconomic status, and home characteristics are predictors of variability in blood

BTEX [23]. The fact that most of the predicted variability in blood BTEX was associated with relatively stable characteristics is reassuring in the context of the limitations of our study design.

Symptoms can be highly sensitive measures of early manifestations of toxicant associated impairments in neurologic function [53]. As such, symptom assessment is appropriate to capture the potentially subtle neurotoxic effects of environmental BTEX levels. Although symptoms are inherently subjective and often less severe than clinically apparent neurologic disease, they are more prevalent in the general population, and offer a valuable metric to measure this public health phenomenon.

In this first study to model the relationship between measured BTEX, individually and as a mixture, and neurologic effects, we observed associations between benzene and CNS symptoms and toluene and PNS symptoms. BTEX are ubiquitous exposures with the potential to meaningfully impact neurologic health at environmental levels experienced by the general population. Our findings highlight the need for future research elucidating timing of BTEX exposure and persistence of neurologic effects, as well as analyzing objective endpoints that may have more direct relevance to neurologic disease progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Environmental blood BTEX levels are associated with neurologic symptoms
- Blood benzene appears to be the agent driving associations in mixture analyses
- Blood benzene is consistently associated with symptoms of central nervous symptom impairment
- Blood toluene may be associated with symptoms of peripheral nervous system impairment

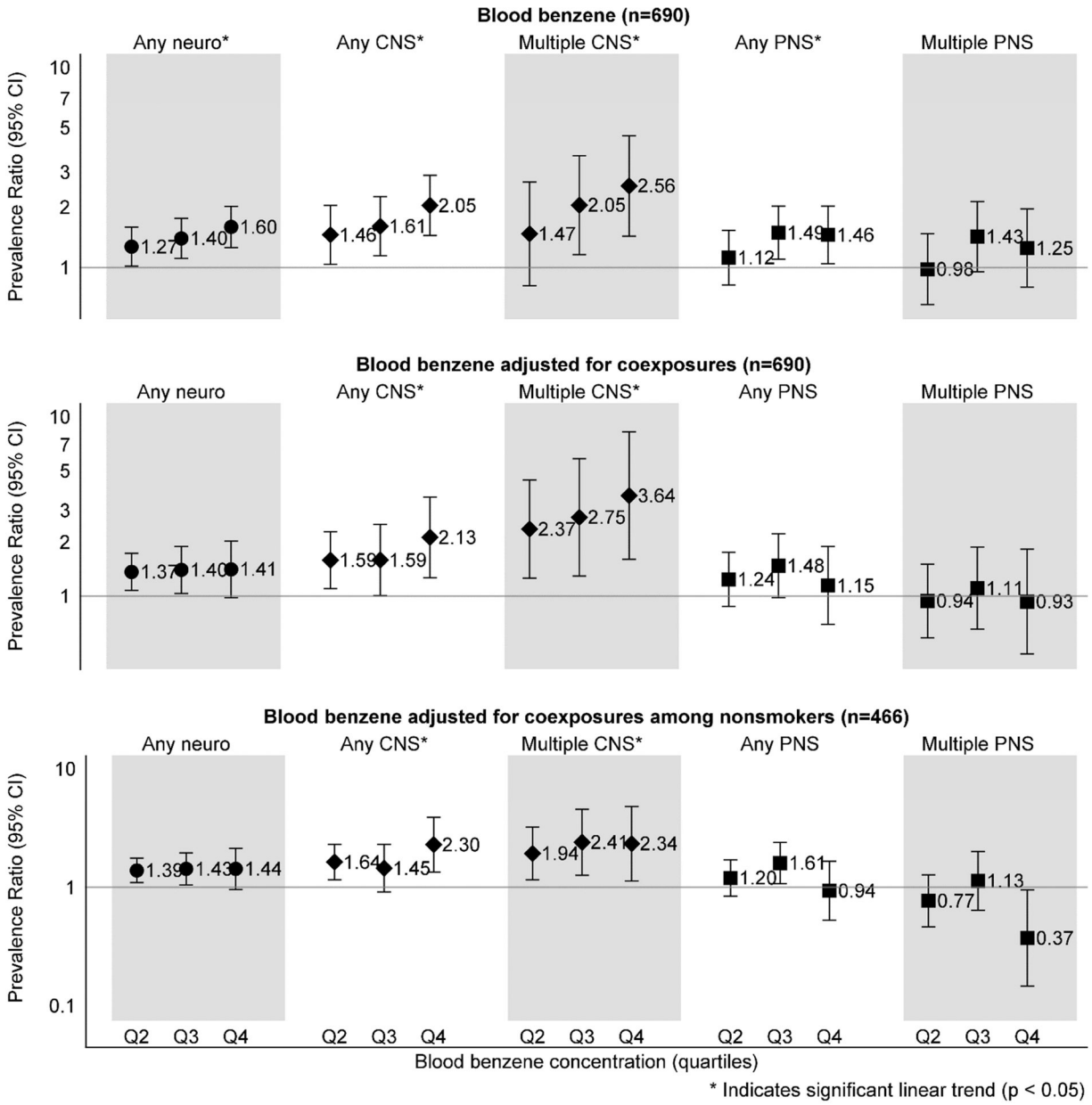

Figure 1.

Individual chemical associations between blood benzene levels and neurologic symptom clusters.

Top panel: adjusted for age, gender, race, education, income, employment status, self-reported smoking, self-reported alcohol consumption, season of enrollment, diabetes diagnosis, maximum THC level, and interval between enrollment and blood draw.

Middle panel: adjusted as in top panel and additionally adjusted for quartiles of toluene, ethylbenzene, and xylenes.

Lower panel: adjusted as in middle panel among self-reported nonsmokers only.

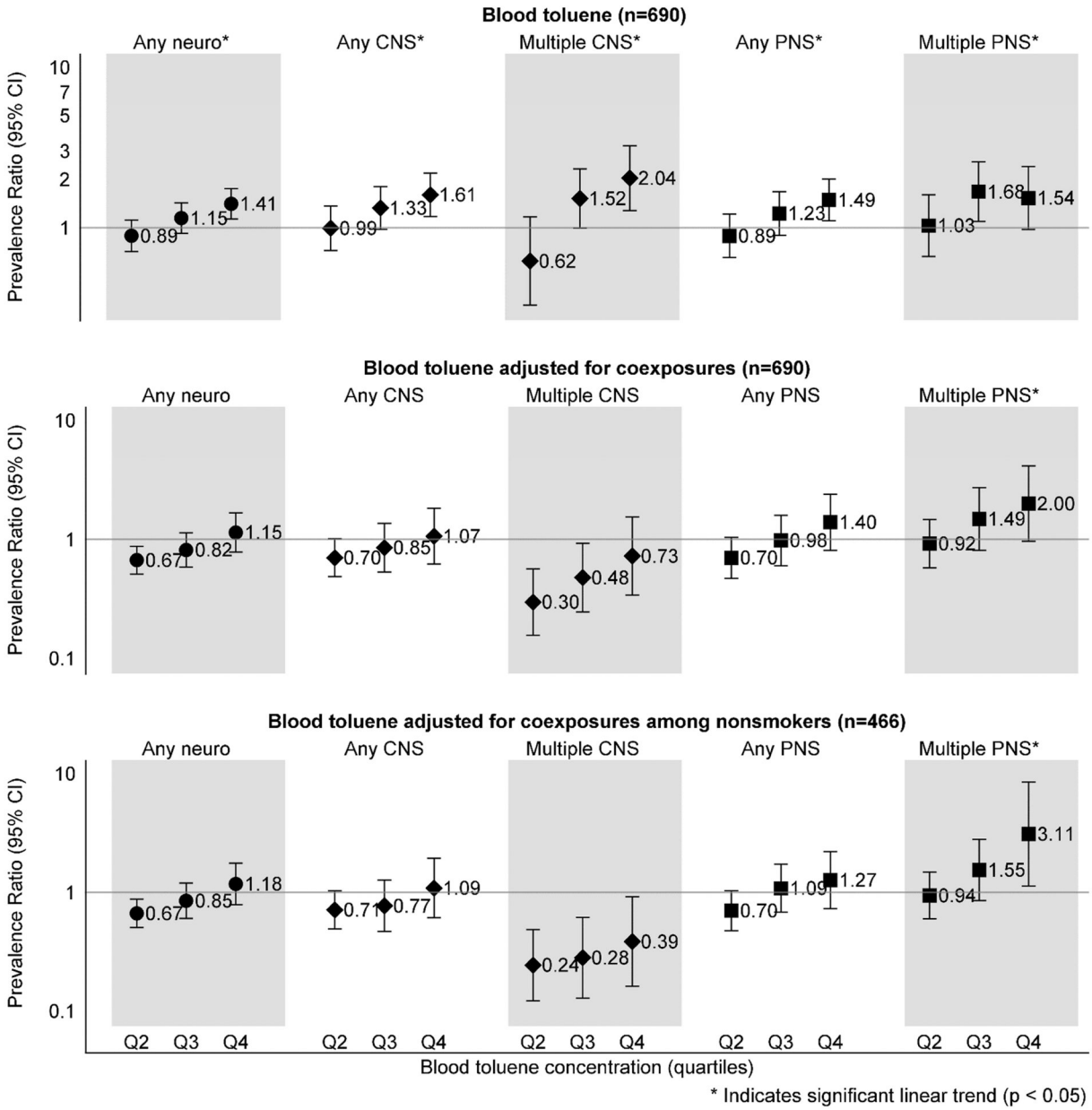

Figure 2.

Individual chemical associations between blood toluene levels and neurologic symptom clusters.

Top panel: adjusted for age, gender, race, education, income, employment status, self-reported smoking, self-reported alcohol consumption, season of enrollment, diabetes diagnosis, maximum THC level, and interval between enrollment and blood draw.

Middle panel: adjusted as in top panel and additionally adjusted for quartiles of benzene, ethylbenzene, and xylenes.

Lower panel: adjusted as in middle panel among self-reported nonsmokers only.

Table 1.

Selected characteristics of analytic population (N=690).

	Overall (N=690)		≥1 neurologic Symptoms (n=340)		No neurologic Symptoms (n=350)	
Characteristics	**N**	**%**	**N**	**%**	**N**	**%**
Male	527	76	246	72	281	80
Age <30 years	141	20	57	17	84	24
30–45	267	39	139	41	128	37
>45	282	41	144	42	138	39
Race						
White	346	50	144	42	202	58
Black	295	43	167	49	128	37
Other race	49	7	29	9	20	6
Diabetes diagnosis	56	8	36	11	20	6
Current smoker	224	32	122	36	102	29
Drinks alcohol	477	69	220	65	257	73
Employed	354	51	152	45	202	58
Completed < high school diploma	151	22	92	27	59	17
High school diploma/GED	258	37	129	38	129	37
Some college	210	30	101	30	109	31
College graduate	71	10	18	5	53	15
Annual income ≤ $20,000	300	43	170	50	130	37
$20,001 – $50,000	253	37	122	36	131	37
> $50,000	137	20	48	14	89	25
Winter enrollment	278	40	143	42	135	39
Spring	126	18	60	18	66	19
Summer	149	22	71	21	78	22
Fall	137	20	66	19	71	20
Maximum total hydrocarbons ≥ 3 ppm	92	13	54	16	38	11
1.0–2.99 ppm	192	28	97	29	95	27
0.3–0.9 ppm	228	33	119	35	109	31
≤ 0.29 ppm	76	11	31	9	45	13
Non-worker	102	15	39	11	63	18
Days between enrollment and blood draw[1]	98		87		112	
Blood benzene, (ng/mL)[1]	0.05		0.09		0.03	
Blood toluene, (ng/mL)[1]	0.18		0.28		0.14	
Blood ethylbenzene, (ng/mL)[1]	0.05		0.06		0.04	
Blood xylenes, (ng/mL)[1]	0.13		0.14		0.12	

[1]Median

Table 2.

Blood BTEX distribution and Spearman rank correlation coefficients (N=690).

	Spearman Correlations			Exposure quartile thresholds (ng/mL)					
	B	**T**	**E**	**Mean ± SD**	**P25**	**P50**	**P75**	**Max**	**>LOD (%)**
Benzene	—			0.13 ± 0.19	0.01	0.05	0.19	1.64	59
Toluene	0.87	—		0.51 ± 3.01	0.08	0.18	0.49	74.8	100
Ethylbenzene	0.77	0.84	—	0.07 ± 0.09	0.02	0.05	0.09	1.48	64
Xylenes	0.75	0.85	0.92	0.21 ± 0.42	0.06	0.13	0.25	7.98	86

SD, standard deviation; P25, 25th percentile; P50, median; P75, 75th percentile; Max, maximum; LOD, limit of detection

Table 3.

Weighted quantile sum regression of neurologic symptoms and one-quartile change in blood BTEX mixture (N=690).

				Mixture weights			
Symptoms	**Prev. (%)**	**OR (95% Cl)**	**√(OR)**	**Benzene**	**Toluene**	**Ethylbenzene**	**Xylenes**
Any neurologic	49	1.47 (1.11, 1.98)	1.21	0.81	0.19		
Any CNS	32	1.40 (1.05, 1.89)	1.18	0.83	0.17		
Multiple CNS	16	1.14 (0.78, 1.67)	1.07	0.87			0.13
Any PNS	31	1.25 (0.93, 1.69)	1.12	0.84	0.05	0.11	
Multiple PNS	20	1.12 (0.78, 1.60)	1.06	0.55	0.36	0.09	

Prev., prevalence of outcome; OR, odds ratio for one-quartile change in BTEX mixture; 95% CI, 95% confidence interval

Mixture weights are constrained from 0 to 1 and sum to 1 for each outcome.

All models adjusted for age, gender, race, education, income, employment status, self-reported smoking, self-reported alcohol consumption, season of enrollment, diabetes diagnosis,

maximum THC level, and interval between enrollment and blood draw.