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Associations between Blood BTEXS Concentrations and Hematologic Parameters among Adult Residents of the U.S. Gulf States

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Abstract

Background—Studies of workers exposed to benzene at average air concentrations below one part per million suggest that benzene, a known hematotoxin, causes hematopoietic damage even at low exposure levels. However, evidence of such effects outside of occupational settings and for other volatile organic compounds (VOCs) is limited.

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Competing Financial Interests Declaration

The authors declare no competing financial interests.

Institutional Review Board Approval

All participants provided verbal informed consent prior to the telephone enrollment interview and written consent prior to the start of the home visit. The study was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences. The Centers for Disease Control and Prevention's (CDC) role was limited to analysis of coded specimens and was determined to not constitute engagement in human subjects research.

Disclaimers

All authors have read and approved the paper. The paper has not been published previously nor is currently being considered by any other peer-reviewed journal. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Color Printing

Color printing is NOT required.

Objective—To investigate associations between ambient exposures to five VOCs, including benzene, and hematologic parameters among adult residents of the U. S. Gulf Coast.

Materials and Methods—Blood concentrations of selected VOCs were measured in a sample of adult participants in the Gulf Long-term Follow-up Study (GuLF STUDY) during 2012 and 2013. Complete blood counts with differentials were also performed on a subset of participants (n=406). We used these data together with detailed questionnaire data to estimate adjusted associations between blood BTEXS (benzene, toluene, ethylbenzene, o-xylene, m/p-xylene, and styrene) concentrations and hematologic parameters using generalized linear models.

Results—We observed inverse associations between blood benzene concentrations and hemoglobin concentration and mean corpuscular hemoglobin concentration, and a positive association with red cell distribution width among tobacco smoke-unexposed participants (n=146). Among tobacco smoke-exposed participants (n=247), we observed positive associations between blood VOC concentrations and several hematologic parameters, including increased white blood cell and platelet counts, suggestive of hematopoietic stimulation typically associated with tobacco smoke exposure. Most associations were stronger for benzene than for the other VOCs.

Conclusions—Our results suggest that ambient exposure to BTEXS, particularly benzene, may be associated with hematologic effects, including decreased hemoglobin concentration, mean corpuscular hemoglobin concentration, and increased red cell distribution width.

1. Introduction

The volatile organic compounds (VOCs) benzene, toluene, ethylbenzene, xylene, and styrene (BTEXS) are important as fuel additives, solvents, and industrial intermediates, and can be found in a diverse array of consumer products. Because these compounds are highly volatile and emission sources are widespread, they are present at detectable concentrations in most human environments, though the most common source of population exposure in the United States is tobacco smoke (ASTDR 2010; Lin et al. 2008; Weisel 2010). Exposures to these compounds are associated with a range of adverse health effects (ATSDR 2007a; Hack et al. 2005; WHO 2000), including hematotoxic effects of benzene exposure (ATSDR 2007a; Galbraith et al. 2010; IARC 2011). Briefly, benzene is metabolized to several reactive metabolites that can damage hematopoietic progenitor cells and lead to depression of the bone marrow (Ross 2000; Snyder and Hedli 1996; Wang et al. 2012). This hematopoietic damage is upstream of blood cell formation and can manifest as reduced blood cell counts (cytopenia) and altered hematologic parameters, while prolonged exposure can lead to hematologic conditions including aplastic anemia, leukemia, and other hematologic cancers (ATSDR 2007a; Galbraith et al. 2010; IARC 2011).

Some (Koh et al. 2015; Lan et al. 2004; Qu et al. 2002), but not all (Collins et al. 1997; Swaen et al. 2010; Tsai et al. 2004), occupational studies have reported decreased blood cell counts and other hematologic abnormalities among workers exposed to benzene even at time-weighted average air concentrations at or below one part per million (ppm). The potential for hematologic effects at low occupational benzene levels raises the possibility that ambient and environmental exposures, though typically lower than occupational exposures, could produce similar effects (Bolden et al. 2015; Brugnone et al. 1998; Smith

2010). Data on hematologic effects of ambient exposure to BTEXS, however, are very limited. In one ecological study, Lee *et al.* observed decreases in multiple blood cell types among children living near a petrochemical production and processing site (n=97) compared to suburban controls (n=95) (Lee et al. 2002). Another ecological study of 158 adult females in Taiwan found associations between distance from a freeway and abnormal white blood cell count (WBC) and hemoglobin concentration; however, the authors observed low correlations between measured air VOC concentrations and distance from freeway, suggesting that the observed associations may be at least partially due to other factors (Jeng et al. 2006). More recently, Pelallo-Martinez *et al.* investigated hematologic effects among children exposed to a mixture of petrochemicals across multiple industrial sites in Mexico (Pelallo-Martinez et al. 2014). The authors observed no differences in hematologic parameters across study sites, representing a range of petrochemical exposures, but did observe negative correlations between certain hematologic parameters (including blood cell counts) and urinary metabolites of benzene and toluene among children from the most highly exposed site (n=20). These studies suggest that ambient VOC exposures may be associated with adverse hematologic effects, though the evidence to date is limited by modest sample sizes, relatively crude quantification of exposure, and little or no control for potential confounding.

This study was carried out in conjunction with the Gulf Long-Term Follow-up Study (GuLF STUDY), a prospective cohort study of individuals who participated in the *Deepwater Horizon* oil spill cleanup and comparison subjects who did not (Kwok et al. (in press)). Blood concentrations of a range of VOCs, including BTEXS, were measured for a sample of Gulf state residents approximately three years after the spill to address community concerns about reports of high levels of these chemicals in some residents. Because BTEXS is rapidly metabolized and excreted (α-phase half-lives of less than an hour) (Ashley et al. 1996; Ashley and Prah 1997), these measurements represent contemporary exposures and not exposures related to clean-up work. Complete blood counts (CBCs) with differentials were also performed on a random subset of study participants. We investigated associations between measured levels of BTEXS in blood and hematologic cell counts and parameters.

2. Methods

2.1 Study Population

The GuLF STUDY is described in detail elsewhere (Kwok et al. (in press)). In brief, GuLF STUDY investigators used administrative records to identify and enroll individuals who were involved in any aspect of *Deepwater Horizon* oil spill work and/or completed worker safety training in anticipation of performing spill-related work. Eligible individuals were at least 21 years of age at time of enrollment and capable of completing an interview in English, Spanish, or Vietnamese. Between March 2011 and March 2013, 32,608 participants completed a detailed telephone enrollment. All cohort members who lived in one of the Gulf states (Alabama, Florida, Louisiana, Mississippi, and eastern Texas) and spoke English or Spanish were invited to participate in a home visit; home visits were completed for 11,193 cohort members between May 2011 and May 2013. The present study includes 406 participants, oversampled for non-smokers, women, and clean-up workers, who provided

additional blood samples during their home visit for measurement of VOCs and CBCs for substudies nested within the main study.

Participants provided written informed consent. The study was approved by the Institutional Review Board of the National Institute of Environmental Health Sciences. The Centers for Disease Control and Prevention's (CDC) role was limited to analysis of coded specimens and was determined to not constitute engagement in human subjects research.

2.2 Home Visit and Biological Specimen Collection

During the home visit, trained field agents interviewed participants about health status, sociodemographic and lifestyle characteristics, and other factors. These agents also collected blood samples that included 10 mL for measurement of VOCs and 2 mL for CBC. Blood samples for VOC measurement were collected using glass blood collection tubes containing potassium oxalate sodium fluoride anticoagulant that were fitted with butyl rubber stoppers, which had been pre-treated by the CDC laboratory to remove VOC residue to minimize pre-collection contamination (Chambers et al. 2005; Chambers et al. 2008). Blood samples were stored in a 4 °C refrigerator prior to being shipped overnight on cold packs to the central study lab, where they were sent in biweekly batches to either the Centers for Disease Control and Prevention in Atlanta, Georgia for VOC analysis or to a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory for CBC analysis.

2.3 Analysis of Biological Samples

Blood samples were analyzed for concentrations of VOCs using previously published methods (Blount et al. 2006; Chambers et al. 2005; Chambers et al. 2006). Briefly, investigators used isotopically labeled analog internal standardization and headspace solid phase microextraction extraction/gas chromatography/mass spectrometry (SPME/GC/MS) to measure concentrations of 46 VOCs, including BTEXS and 2,5-dimethylfuran (2,5-DMF; a biomarker of exposure to tobacco smoke) (Chambers et al. 2011; Charles et al. 2008). Limit of quantitation was defined as the concentration where both blank-level sample false positive and detection-level sample false negative results fell below 5% (Armbruster and Pry 2008). A portion of ethylbenzene measurements were affected by a coeluting column-bleed interferent and were excluded. We excluded one participant with multiple aberrantly high blood VOC concentrations from this analysis. For all statistical analyses, we imputed blood VOC concentrations below the limit of detection (LOD) as the LOD divided by the square root of two (Lubin et al. 2004) and natural log (ln)-transformed blood BTEXS concentrations.

A central CLIA-certified laboratory carried out complete blood counts with leukocyte differential using routine clinical laboratory procedures. The following hematologic parameters were measured: red blood cell count (RBC, ×10E6/uL), hemoglobin concentration (g/dL), hematocrit (%), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin concentration (MCHC, g/dL), red cell distribution width (RDW, %), platelets (×10E6/uL), white blood cell count (WBC, ×10E3/uL), neutrophils (%), lymphocytes (%), and monocytes (%), and eosinophils (%). Derived hematologic parameters include hematocrit (the ratio of the volume of red blood cells to total blood volume), MCHC

(the quotient of hemoglobin concentration divided by hematocrit), and RDW (a measure of variability of RBC size, equal to the standard deviation of the RBC corpuscular volume divided by the MCV and multiplied by 100 to obtain a percentage). We also calculated leukocyte counts by cell subtype by multiplying total WBC count by the proportion of each subtype. We ln-transformed total WBC count and WBC subtype counts to improve normality. Some participants had eosinophil percentages equal to zero that could not be ln-transformed; for these we imputed eosinophil values as 0.5% prior to ln-transform.

2.4 Statistical Analyses

We generated descriptive statistics to characterize the population and the blood BTEXS concentrations, including correlations between individual compounds and comparisons to values measured in adult participants (≥21 years) of NHANES 2005–2008 (which were closest in time to the GuLF STUDY samples). For our primary analyses, we used Generalized Linear Models to estimate linear changes in hematologic parameters per unit change in ln-transformed blood BTEXS concentration. We adjusted all models for the following variables identified on the basis of a directed acyclic graph to account for factors potentially related to both BTEXS levels and hematologic parameters (Greenland et al. 1999; Rothman 2008): sex (male, female), race (White, Black, Other), age in years at home visit (<35, 35–50, >50), Body Mass Index at home visit (BMI, kg/m^2; <18.5–24.9, 25.0–29.9, >30), weekly alcohol consumption assessed at enrollment (not current drinker, fewer than 7 drinks per week, 7 or more drinks per week), educational attainment at enrollment (less than high school or equivalent, high school diploma or GED, some college or 2 year degree, 4 year college graduate or more), and annual household income at enrollment (< $20,000, $20,000–$50,000, >$50,000). Because tobacco smoke is an important source of VOC exposure that also exerts hematologic effects through non-VOC pathways, we conducted all analyses stratified by exposure to tobacco smoke (unexposed, exposed). To classify tobacco smoke exposure, we used self-report of primary smoking status and daily passive tobacco smoke exposure, and a biomarker of tobacco smoke exposure (2,5-DMF). Tobacco smoke-unexposed participants (n=146) reported not currently smoking and less than 30 minutes of daily passive tobacco smoke exposure, and also had blood 2,5-DMF concentrations less than or equal to 14 ng/L; this value was identified by Chambers et al. (2011) to discriminate between daily and less-than-daily smokers. Conversely, tobacco smoke-exposed participants (n=247) reported at least some current cigarette smoking, 30 minutes or more of daily passive tobacco exposure, or had blood 2,5-DMF concentrations greater than 14 ng/L. We omitted 13 participants missing necessary data on smoking status. We evaluated associations among tobacco smoke-exposed participants both with and without adjustment for degree of tobacco smoke exposure, including combinations of primary tobacco smoke exposure (not current smoker, 10 or fewer cigarettes daily, more than 10 cigarettes daily), daily passive tobacco smoke exposure (less than 30 minutes, 30 or more minutes), and ln-transformed blood 2,5-DMF concentrations. We preferentially used participants' interview responses from time of home visit (and blood draw), but used related information from enrollment when home visit data were not available.

In sensitivity analyses, we used categorical specifications of blood BTEXS concentrations to investigate nonlinear trends: we either used tertiles or, if more than one third of values were

below the LOD, we classified participants as either below the LOD or into approximately equally sized groups above or below the median of values above the LOD. We also investigated potential impacts of oil spill clean-up participation on both blood BTEXS concentrations and hematologic parameters by estimating adjusted associations between these parameters and an indicator variable for intensity of exposures experienced during cleanup, created using the GuLF STUDY Job Exposure Matrix (Kwok et al. (in press)), which assigned participants a level of exposure based on their reported experiences during cleanup (Non-Worker/Very Low/Low/Medium/High). In this analysis, we compared participants with Low, Very Low, and Non-Worker exposure classifications (n=197) to participants with Medium and High exposure classification (n=208). We adjusted these associations for sex, race, age in years at home visit, Body Mass Index at home visit, weekly alcohol consumption assessed at enrollment, and self-reported occupational exposure to any of the following BTEXS-related materials in the 24 hours prior to blood draw (unexposed (n=351)/exposed (n=44)): gasoline or diesel engine exhaust, degreasers or chemicals used to clean metal parts, metal machining oils, or paints, varnishes, stains or strippers. This adjustment set was identified on the basis of two Directed Acyclic Graphs of the associations between cleanup-related exposures and BTEXS and cleanup-related exposures and hematologic parameters, and is a sufficient adjustment set for each set of associations.

We considered p-values below 0.05 to be statistically significant. We conducted all analyses using SAS 9.4 software (SAS Inc., Cary, NC).

3. Results

3.1 Study Population

Table 1 provides demographic and lifestyle characteristics of the study participants. This population was mostly male (75%), relatively young (median age: 41 years), racially diverse (52% non-White), with almost half obese (48% had BMI >30). Most participants reported their highest educational attainment as a high school diploma (39%) or less (25%). Most participants reported less than $50,000 annual household income, with about 42% reporting less than $20,000. Although we oversampled nonsmokers, 34% of participants reported currently smoking and 34% reported 30 or more minutes per day of passive tobacco smoke exposure. Most participants (95%) lived in a county adjacent to the Gulf coast, and most (89%) had worked on the oil spill. Most home visits occurred within one year of enrollment (93%), and 93% of visits occurred more than one year after last reported participation in cleanup-related activities.

3.2 Blood BTEXS Concentrations

Table 2 provides descriptive statistics of blood BTEXS concentrations and values from adult participants of NHANES 2005–2008 for comparison. Blood BTEXS concentrations were approximately log-normally distributed with long right tails. A substantial proportion of measurements were below the instrumental limits of detection (LOD) for all compounds except for toluene; as expected, tobacco smoke-exposed participants were more likely to have detectable BTEXS concentrations than tobacco smoke-unexposed participants. Blood benzene, toluene, ethylbenzene, and m/p-xylene concentrations were comparable between

our study population and NHANES participants with blood 2,5-DMF concentrations below 14 ng/L; conversely, blood m/p-xylene concentrations were notably lower and blood styrene concentrations were notable higher in our population than among these NHANES participants. Tobacco smoke-exposed participants had generally lower blood BTEXS concentrations than NHANES participants with blood 2,5-DMF concentrations greater than 14 ng/L, with the exception of styrene. Because these discrepancies may have resulted from including persons in our smoke-exposed strata with blood 2,5-DMF concentrations below 14 ng/L if they had reported smoking or passive smoke exposure even without blood 2,5-DMF concentrations below 14 ng/L, we examined BTEXS levels among GuLF STUDY participants using only blood 2,5-DMF concentrations as our smoking strata criterion (less than 14 ng/L, greater than or equal to 14 ng/L). In this comparison, we observed that the geometric means of blood BTEXS concentrations in GuLF STUDY participants with blood 2,5-DMF concentrations below 14 ng/L were comparable to the NHANES participants when using this alternative criteria, with the exception of styrene which remained higher among GuLF STUDY participants. Conversely, 95th percentile of blood BTEXS were often higher among GuLF STUDY participants than NHANES values using this classification. We observed comparable levels of BTEXS compounds between the GuLF STUDY and NHANES among participants with blood 2,5-DMF concentrations greater than or equal to 14 ng/L, again with the exception of styrene (data not shown). Correlations between compounds were weak to strong among tobacco smoke-unexposed participants (Spearman correlation coefficients: 0.18 to 0.84), but more strongly correlated among tobacco smoke-exposed participants (Spearman correlation coefficients: 0.55 to 0.93).

3.3 Associations between Blood BTEXS Concentrations and Hematologic Parameters

Table 3 presents adjusted estimates for linear changes in hematologic parameters per unit increase in ln-transformed blood BTEXS concentrations, including effect estimates (e.g., β), standard errors, and Wald p-values. Among tobacco smoke-unexposed participants, we observed inverse associations between blood benzene concentrations and hemoglobin concentration and mean corpuscular hemoglobin concentration (MCHC), and a positive association with RDW. Associations with these hematologic parameters were stronger for benzene than for other compounds. Blood concentrations of toluene, o-xylene, and m/p-xylenes, and, to a lesser degree, benzene and ethylbenzene, were inversely associated with platelet counts. Blood concentrations of o- and m/p-xylene only were inversely associated with hematocrit. We observed generally null or weak inverse associations between blood BTEXS concentrations and mean corpuscular volume (MCV); styrene was the only compound for which the association approached statistical significance. We observed no apparent associations between blood BTEXS concentrations and either WBC or RBC counts among tobacco-unexposed participants.

Among tobacco smoke-exposed participants, we observed positive associations of blood benzene concentrations with RBC and WBC counts, hemoglobin concentration, and hematocrit, MCV, and MCHC. We also observed a weak (non-significant) inverse association with red cell distribution width and similarly overserved a weak positive association with platelet count. We observed similar associations between these hematologic parameters and toluene, ethylbenzene, and o- and m/p-xylene. Adjustment for degree of

tobacco smoke exposure among tobacco smoke-exposed participants reduced precision and attenuated most associations (data not shown). Effect estimates were more precise among tobacco smoke-exposed participants than among tobacco smoke-unexposed participants, due at least in part to the larger number of tobacco smoke-exposed participants.

Supplemental Table 1 presents the results of categorical analyses, with point estimates and standard errors for change in hematologic parameter by quantile of blood BTEXS concentration. Trends among tobacco smoke-unexposed participants were often consistent with the primary linear results, though some associations demonstrated potentially non-monotonic trends. Small quantile sizes among tobacco smoke-unexposed participants resulted in relatively imprecise point estimates. Among tobacco smoke-exposed participants, trends across quantiles of blood BTEXS concentrations were often monotonic and in the same direction as our primary results.

As expected, we did not observe any strong associations between intensity of cleanup-related exposures and either blood VOC concentrations or hematologic parameters in these blood samples (all p-values > 0.05), which were collected approximately three years after the spill (data not shown).

4. Discussion

4.1 Summary and Interpretation of Results

In this cross-sectional analysis, blood benzene concentrations were inversely associated with hemoglobin concentration and mean corpuscular hemoglobin concentration, and positively associated with red cell distribution width, among tobacco smoke-unexposed participants. Conversely, among tobacco smoke-exposed participants, we observed positive associations between blood benzene concentrations and several hematologic parameters, including RBC and WBC counts. Associations tended to be stronger for benzene than for the other compounds, which is consistent with previous evidence of these chemicals' hematotoxicity. To our knowledge, ours is the largest study to date to employ biological measures of BTEXS exposures in the investigation of hematologic effects of environmental BTEXS exposures.

Current mechanistic evidence supports the possibility of hematologic effects of low dose exposures to VOCs, particularly benzene. The possible mechanisms have been extensively studied (Ross 2000; Smith 2010; Snyder and Hedli 1996; Wang et al. 2012): briefly, benzene that is metabolized in the liver and bone marrow is formed into multiple reactive metabolites (including benzene oxide, phenol, quinones, and muconic acid) that exhibit cytotoxicity to both hematopoietic progenitor cells and also mature blood cells (Hirabayashi and Inoue 2010; Ross 1996; Wang et al. 2012). This cytotoxicity leads to depression of the hematopoietic tissue that can manifest as reduced blood cell counts, among other effects. There is additional evidence that production of cytotoxic metabolites may exhibit a non-linear exposure-response, such that lower benzene exposures produce proportionately more reactive metabolites than higher exposures (Kim et al. 2006a; Kim et al. 2006b; Rappaport et al. 2005; Rappaport et al. 2009; Rappaport et al. 2013). Together, current mechanistic understanding supports the plausibility of hematologic effects of low dose exposure to benzene and other VOCs.

Most observational evidence of low dose VOC-related hematotoxicity comes from occupational studies among workers exposed to benzene at air concentrations below one ppm per 8-hour time-weighted average, which is the current Permissible Exposure Limit mandated by the U.S. Occupational Safety and Health Administration. For instance, in a longitudinal study of benzene-exposed workers in China, Qu *et al.* observed statistically significant decreases in multiple blood cell counts among the lowest exposed workers (<0.25 ppm 4-week average) compared to unexposed controls (Qu et al. 2002). Similarly, in a cohort study of benzene-exposed workers in China, Lan *et al.* observed decreases in all types of white blood cells and platelets among the lowest exposed workers (<1 ppm) compared to referents (Lan et al. 2004). More recently, Koh *et al.* used the Korean Special Health Examination Database to assign exposure levels to benzene-exposed workers using process, factory, and industry codes (Koh et al. 2015); the authors reported decreased RBCs among male workers exposed to 0.01–0.1 ppm and 0.1–0.5 ppm, compared to the referent group (<0.01 ppm). Three industry-supported occupational studies by Swaen *et al.*, Collins *et al.*, and Tsai *et al.*, however, did not report such effects among workers exposed to benzene at air concentrations below one ppm (Collins et al. 1991; Swaen et al. 2010; Tsai et al. 2004). This issue, while unresolved, raises concerns about the hematologic effects of low-level benzene exposure among the general population.

Current evidence relating environmental exposures to VOCs to alterations in hematologic parameters is limited. One ecologic study observed lower counts of multiple blood cell types among children living near a petrochemical region compared to suburban controls (Lee et al. 2002). A similar study of female residents of a large city observed apparent associations between distance from a large freeway and abnormal leukocyte count and hemoglobin concentration (Jeng et al. 2006). More recently, another study observed negative correlations between certain hematologic parameters (including hemoglobin concentration and hematocrit) and urinary biomarkers of benzene and toluene exposure among children living near petrochemical operations (n=20) (Pelallo-Martinez et al. 2014). This limited literature suggests that environmental exposures to VOCs may be associated with hematologic parameters; however, these studies had modest sample sizes, relied primarily upon ecologic exposure characterization, and had limited ability to adjust for potential confounding factors.

In our study, we observed that associations between blood BTEXS concentrations and hematologic parameters were strongly influenced by the extent of tobacco smoke exposure in the analysis population. Specifically, many of the associations we observed among tobacco smoke-exposed participants were in the opposite direction of the expected hematopoietic suppressive effects of benzene exposure, but were concordant with hematopoietic stimulating effects of tobacco smoke, a potent source of VOC exposure. Tobacco smoke is a complex mixture of many compounds, including benzene, that produce stimulating effects on hematopoietic tissue and the immune system, which lead to alterations in hematologic parameters, including increasing white blood cell counts, mean corpuscular volume, and hemoglobin concentration (Andrews and Tingen 2006; Wannamethee et al. 2005; Yanbaeva et al. 2007); consequently, the associations we investigated were vulnerable to confounding by non-VOC constituents and effects of tobacco smoke. In our primary analyses among tobacco smoke-exposed participants, we observed that many of the associations between blood BTEXS concentrations and hematologic parameters reflected

known hematologic effects of tobacco smoke exposure, despite adjustment for combinations of self-reported current smoking status and daily passive smoke exposure and blood 2,5-DMF concentrations. Consequently, we ultimately opted to stratify analyses by exposure to tobacco smoke using sensitive criteria to identify a population with minimal exposure to tobacco smoke. Specifically, tobacco smoke-unexposed participants were those who reported not currently smoking and fewer than 30 minutes of daily passive tobacco smoke exposure and also had blood 2,5-DMF concentrations below 14 ng/L. In choosing these criteria, we sought to both maximize the sample size of our tobacco smoked-unexposed strata and minimize degree of exposure to tobacco smoke. We expect associations among this population to more accurately reflect non-tobacco VOC exposures and be less vulnerable to confounding by tobacco smoke. When restricted to this population, many of the associations we observed among the tobacco smoke-exposed participants attenuated, and some reversed direction, though this may be at least partially attributed to the smaller size of the analysis population. Specifically, among tobacco smoke-unexposed participants, we observed that blood benzene concentrations were inversely associated with hemoglobin concentration and mean corpuscular concentrations, and positively associated with red cell distribution width. Other studies have also reported inverse associations between environmental VOC exposure and hemoglobin concentrations (Lee et al. 2002; Pelallo-Martinez et al. 2014), and increased risk of abnormal hemoglobin concentration (Jeng et al. 2006). To our knowledge, no other studies of low-dose occupational exposures or ambient exposures to VOCs have reported on associations with mean corpuscular hemoglobin concentration (MCHC) or red cell distribution width (RDW), and additional study is needed to substantiate these associations. While we observed only modest associations suggestive of potentially hematotoxic effects, our findings contribute to previous evidence that ambient and low dose benzene exposure may be associated with impairment of the hematopoietic system.

4.2 Strengths and Limitations

The results of our study need be interpreted in light of our study's strengths and limitations. One limitation is that the study population included mostly young to middle-aged males and over a third of participants reported current cigarette smoking, which reduced our ability to investigate associations among females and led to less precise effect estimates among tobacco smoke-unexposed participants. Also, while it is possible this population's experience with the oil spill influenced the associations we observed, this is unlikely given the biological half-lives of VOCs and the recovery period for benzene-induced hematotoxicity. Specifically, VOCs are typically eliminated from the body within hours to days, and available evidence suggests hematopoietic suppressive effects of benzene exposure typically resolve within a few months after exposure ceases (ATSDR 2007a). Among participants who worked on the oil spill, over 95% of their home visits, when the blood samples for this study were collected, occurred at least 180 days after each participant's last reported work on the spill. In addition, all blood samples were collected more than two years after the well was sealed (Operational Science Advisory Team 2010). In a supplementary analysis, we observed no apparent differences in blood BTEXS concentrations or hematologic parameters between those who participated in oil spill work and those who did not.

Additionally, a high proportion of participants had blood BTEXS concentrations below our instrumental LOD, and we used single imputation to assign values to these participants; this approach may introduce bias and lead to overly conservative standard errors when substantial proportions of participants (e.g., >5%) have values above the LOD (Lubin et al. 2004). Second, blood BTEXS concentrations were moderately to strongly correlated with one another and our sample sizes were modest, which limited our ability to adjust for co-exposures that have been shown to modify the hematotoxic effects of benzene exposure (Medinsky et al. 1994; Ross 2000). We attempted to address this in secondary analyses by additionally adjusting each model for a single co-exposure and observed potential interactions between benzene and other VOCs, though these associations were highly imprecise. Third, our analyses were cross-sectional, with both blood BTEXS concentrations and CBCs measured at the same time. Current evidence suggests that benzene primarily affects progenitor cells (Hirabayashi and Inoue 2010; Lan et al. 2004; Wang et al. 2012) and the measured blood BTEXS concentrations may not reflect levels during the most etiologically relevant window. While there is limited evidence pertaining to temporal variability of benzene exposures outside of occupational settings, personal activities and proximate exposures appear to exert a much greater influence on VOC exposures than do outdoor or ambient concentrations (Wallace 1989; Wallace et al. 1989; Wallace 1995). Any exposure measurement error in this study would likely be non-differential and would tend to attenuate observed associations. Future studies of ambient BTEXS-related hematotoxicity would benefit by measuring blood BTEXS concentrations in a more etiologically targeted window(s) prior to hematologic assessment, ideally at multiple time points.

Most of our statistical analyses assumed a linear association between blood BTEXS concentrations and hematologic parameters, though some evidence supports non-linear associations, particularly at low doses (Kim et al. 2006a; Kim et al. 2006b; Rappaport et al. 2005; Rappaport et al. 2009; Rappaport et al. 2013). However, most associations appeared to be monotonic when we assessed trends by categorical analysis. Finally, as an observational study, the associations we observed may have been at least partially attributable to residual confounding and/or chance.

Our study also possesses notable strengths. The GuLF STUDY collected a large body of information among a comparatively large and diverse population. This information included measurements of blood BTEXS concentrations, hematologic parameters, and detailed interview data on a range of relevant demographic and lifestyle factors. Our blood VOC concentrations were measured by the laboratory that conducts all NHANES VOC analyses. Our outcomes, the hematologic parameters represented by CBCs and measured in a CLIA-certified laboratory, have been associated with hematologic effects of BTEXS exposures in occupational settings, including at low doses (Koh et al. 2015; Lan et al. 2004; Qu et al. 2002), and have also been used in a few environmental studies (Jeng et al. 2006; Lee et al. 2002; Pelallo-Martinez et al. 2014). Additionally, relevant interview data enabled us to adjust for a range of potential confounding factors and also assess confounding by tobacco smoke exposure, a particularly important confounding variable, in multiple ways, including self-report of primary and passive smoking status in conjunction with a biomarker of tobacco smoke.

5. Conclusions

We used data collected from GuLF STUDY participants to investigate hematologic effects of ambient VOC exposures among a relatively understudied population of adults living in Gulf coast states. We observed inverse associations between blood benzene concentrations and hemoglobin concentration and mean corpuscular hemoglobin concentration, and a positive association with red cell distribution width among tobacco smoke-unexposed participants. Conversely, we found that blood benzene concentrations were positively associated with multiple hematologic parameters among participants with tobacco smoke exposure. The stronger associations we typically observed for benzene than for the other studied VOCs is consistent with previous research. Our results contribute to previous evidence that exposure to low doses of benzene, and possibly of other VOCs, may be associated with adverse hematologic effects, including depression of the hematopoietic tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

25-DMF	2,5-dimethylfuran
BTEXS	benzene, toluene, ethylbenzene, xylene, and styrene
CBC	complete blood count
CDC	Centers for Disease Control
CLIA	Clinical Laboratory Improvement Amendments
Est	effect estimate
GuLF STUDY	Gulf Long-term Follow-up Study
LOD	limit of detection
MCHC	mean corpuscular hemoglobin concentration
ln	natural log
MCV	mean corpuscular volume
NHANES	National Health and Nutrition Examination Survey
ppm	parts per million

RBC	red blood cell count
RDW	red cell distribution width
SE	standard error
VOCs	volatile organic compounds
WBC	white blood cell count

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Highlights

- Among tobacco smoke-unexposed participants, we observed inverse associations between blood benzene concentrations and hemoglobin concentration and mean corpuscular hemoglobin concentration, and a positive association with red cell distribution width.
- Among tobacco smoke-exposed participants, we observed positive associations between blood VOC concentrations and several hematologic parameters, including increased white blood cell and platelet counts, suggestive of hematopoietic stimulation typically associated with tobacco smoke exposure.
- Most associations with were stronger for benzene than for the other VOCs.

Table 1

Demographic and lifestyle characteristics.

Characteristic		Full Population (n=406)	Tobacco Smoke-Unexposed* (n=146)	Tobacco Smoke-Exposed** (n=247)
Age in years	<35	143 (35)	40 (27)	96 (39)
	35–50	147 (36)	58 (40)	86 (35)
	>50	115 (28)	48 (33)	65 (26)
Sex	Male	306 (75)	106 (73)	194 (79)
	Female	100 (25)	40 (27)	53 (21)
Race	White	195 (48)	68 (47)	122 (49)
	Black	178 (44)	61 (42)	110 (45)
	Other	32 (8)	17 (12)	15 (6)
Body Mass Index (kg/m^2)	Underweight (17.3–18.5)	6 (1)	0 (0)	6 (2)
	Normal weight (18.5–24.9)	73 (18)	19 (13)	51 (21)
	Overweight (25.0–29.9)	132 (33)	45 (31)	82 (34)
	Obese (29.9–52.1)	191 (48)	81 (56)	105 (43)
Highest educational attainment	Less than high school or equivalent	102 (25)	34 (23)	64 (26)
	High school diploma or GED	159 (39)	48 (33)	106 (43)
	Some college or 2 year degree	106 (26)	42 (29)	62 (25)
	4 year college graduate	38 (9)	22 (15)	15 (6)
Annual household income	Less than $20,000	161 (42)	51 (38)	101 (42)
	$20,001 to $50,000	130 (34)	41 (30)	89 (37)
	More than 50,000	95 (25)	44 (32)	48 (20)
Cigarette smoking status	Not current smoker	266 (66)	146 (100)	108 (44)
	Current smoker, fewer than 10 cigarettes per day	75 (19)	0 (0)	75 (31)
	Current smoker, 10 or more cigarettes per day	61 (15)	0 (0)	61 (25)
Daily passive smoke exposure	Fewer than 30 minutes	262 (66)	146 (100)	107 (43)
	30 minutes or more	140 (34)	0 (0)	140 (57)
Alcohol consumption	Not current drinker	125 (32)	47 (34)	74 (31)
	Currently drinks less than 7 drinks per week	199 (52)	71 (51)	120 (51)
	Currently drinks 7 or more drinks per week	62 (16)	20 (14)	42 (18)

*Tobacco smoke-unexposed participants reported no primary tobacco use, less than 30 minutes of passive smoke exposure, and had blood 2,5-DMF concentrations less than 14 ng/L.

**Tobacco smoke-exposed participants reported primary tobacco use, 30 or more minutes of passive smoke exposure, or had blood 2,5-DMF concentrations greater than or equal to 14 ng/L.

Table 2

Blood BTEXS concentrations (ng/L).

							NHANES 2005–08 Values*		GuLF STUDY Spearman Correlation Coefficients					
Population	**Compound**	**N**	**LOD**	**%>LOD**	**GM**	**P95**	**GM**	**P95**	**Benzene**	**Toluene**	**Ethylbenzene**	**o-Xylene**	**m/p-Xylene**	**Styrene**
Tobacco Smoke-Unexposed**	Benzene	142	24	35	25	84	22	56	1	0.36	0.28	0.18	0.25	0.27
	Toluene	144	25	99	88	314	86	329		1	0.54	0.47	0.63	0.29
	Ethylbenzene	105	24	39	26	104	26	75			1	0.70	0.77	0.62
	o-Xylene	142	24	39	25	99	28	84				1	0.84	0.27
	m/p-Xylene	146	34	74	61	390	85	259					1	0.36
	Styrene	141	30	59	52	882	25	55						1
Tobacco Smoke-Exposed***	Benzene	241	24	75	91	526	188	561	1	0.88	0.79	0.59	0.74	0.56
	Toluene	244	25	100	262	1170	478	1393		1	0.83	0.75	0.86	0.55
	Ethylbenzene	182	24	77	56	199	83	210			1	0.86	0.92	0.69
	o-Xylene	237	24	71	37	113	54	135				1	0.93	0.55
	m/p-Xylene	241	34	93	127	426	226	585					1	0.55
	Styrene	234	30	87	98	1110	92	233						1

Abbreviations: GuLF STUDY, Gulf Long-term Follow-up Study, NHANES, National Health and Nutrition Examination Survey; LOD, Limit of Detection; GM, Geometric Mean; P95, 95th percentile.

* NHANES values for Tobacco Unexposed reflect adult NHANES participants (≥ 21 years) with blood 2,5-DMF concentrations less than 14 ng/mL; values for Tobacco Exposed reflect NHANES participants with blood 2,5-DMF concentrations greater than or equal to 14 ng/L.

** Tobacco smoke-unexposed participants reported no primary tobacco use, less than 30 minutes of passive smoke exposure, and had blood 2,5-DMF concentrations less than 14 ng/L.

*** Tobacco smoke-exposed participants reported primary tobacco use, 30 or more minutes of passive smoke exposure, or had blood 2,5-DMF concentrations greater than or equal to 14 ng/L.

Table 3

Estimated linear changes in hematologic parameters per unit increase in ln-transformed blood BTEXS concentrations (ng/L).

Population	Hematologic Parameter	Benzene (n=142)			Toluene (n=144)			Ethylbenzene (n=106)			o-Xylene (n=142)			m/p-Xylene (n=146)			Styrene (n=141)		
		Effect Estimate	Standard Error	P-Value	Effect Estimate	Standard Error	P-Value	Effect Estimate	Standard Error	P-Value	Effect Estimate	Standard Error	P-Value	Effect Estimate	Standard Error	P-Value	Effect Estimate	Standard Error	P-Value
Tobacco Smoke- Unexposed**	RBC (×10E6/uL)	−0.01	0.06	0.8	−0.03	0.05	0.6	0.01	0.07	0.9	−0.10	0.06	0.10	−0.1	0.04	0.2	0.04	0.03	02
	Hemoglobin (g/dL)	−0.36	0.14	0.01	0.04	0.14	0.8	−0.15	0.17	0.4	−0.32	0.15	0.03	−0.17	0.11	0.1	−0.03	0.07	0.7
	Hematocrit (%)	−0.39	0.42	0.3	−0.05	0.39	0.9	−0.26	0.50	0.6	−1.16	0.42	0.01	−0.74	0.31	0.02	0.02	0.22	0.9
	Mean Corpuscular Volume (fL)	−1.07	0.72	0.14	0.31	0.69	0.7	−0.92	0.88	0.3	−0.80	0.75	0.3	−0.75	0.56	0.18	−0.68	0.37	0.07
	Mean Corpuscular Hemoglobin Concentration (g/dL)	−0.39	0.14	0.01	0.10	0.14	0.5	−0.06	0.15	0.7	0.16	0.15	0.3	0.22	0.11	0.05	−0.05	0.08	0.5
	Red Cell Distribution Width (%)	0.38	0.14	0.01	−0.09	0.13	0.5	0.02	0.16	0.9	−0.13	0.14	0.4	−0.13	0.11	0.2	0.07	0.07	0.3
	Platelets (×10E6/uL)	−8.74	8.04	0.3	−18.66	7.36	0.01	−16.73	9.49	0.08	−17.03	8.19	0.04	−14.28	6.10	0.02	−6.08	4.22	0.15
	WBC (×10E3/uL) *	0.04	0.04	0.3	0.00	0.04	0.9	0.04	0.04	0.4	0.01	0.04	0.8	0.00	0.03	0.9	0.02	0.02	0.4
	Neutrophils (×10E3/uL) *	0.04	0.05	0.4	0.03	0.05	0.6	0.11	0.06	0.07	0.03	0.06	0.6	0.03	0.04	0.4	0.02	0.03	0.4
	Lymphocytes (×10E3/uL) *	0.03	0.04	0.4	−0.04	0.03	0.2	−0.03	0.04	0.4	−0.02	0.04	0.6	−0.03	0.03	0.2	0.02	0.02	0.3
	Monocytes (×10E3/uL) *	−0.04	0.06	0.5	0.01	0.05	0.9	0.07	0.05	0.2	0.05	0.06	0.4	0.05	0.04	0.19	0.04	0.03	0.12
	Eosinophils (×10E3/uL) *§	0.16	0.09	0.07	0.01	0.09	0.9	−0.09	0.11	0.4	−0.05	0.09	0.6	0.00	0.07	1.0	0.05	0.05	0.3
Tobacco Smoke-Exposed***	RBC (×10E6/uL)	0.06	0.03	0.02	0.09	0.03	0.006	0.10	0.04	0.02	0.10	0.04	0.02	0.10	0.04	0.01	0.02	0.03	0.5
	Hemoglobin (g/dL)	0.39	0.07	<.0001	0.43	0.08	<.0001	0.44	0.12	0.0002	0.47	0.12	0.0001	0.47	0.11	<.0001	0.15	0.08	0.05
	Hematocrit (%)	0.98	0.19	<.0001	1.11	0.22	<.0001	1.25	0.30	<.0001	1.23	0.32	0.000	1.25	0.28	<.0001	0.41	0.20	0.04
	Mean Corpuscular Volume (fL)	1.02	0.38	0.01	0.83	0.45	0.06	0.80	0.52	0.12	0.72	0.65	0.3	0.90	0.55	0.10	0.59	0.38	0.12
	Mean Corpuscular Hemoglobin Concentration (g/dL)	0.15	0.06	0.01	0.16	0.07	0.02	0.07	0.10	0.5	0.16	0.10	0.11	0.13	0.09	0.12	0.04	0.06	0.5
	Red Cell Distribution Width (%)	−0.11	0.07	0.09	−0.10	0.08	0.2	−0.14	0.11	0.19	−0.22	0.11	0.04	−0.13	0.10	0.17	−0.05	0.07	0.5
	Platelets (×10E6/uL)	5.85	3.93	0.14	4.23	4.48	0.3	5.76	6.48	0.4	−1.36	6.46	0.8	3.77	5.50	0.5	−1.56	4.01	0.7
	WBC (×10E3/uL) *	0.10	0.02	<.0001	0.10	0.02	<.0001	0.10	0.03	0.0004	0.11	0.03	0.0002	0.10	0.02	<.0001	0.03	0.02	0.08
	Neutrophils (×10E3/uL) *	0.11	0.02	<.0001	0.11	0.03	0.000	0.10	0.04	0.01	0.11	0.04	0.01	0.09	0.03	0.01	0.04	0.03	012
	Lymphocytes (×10E3/uL) *	0.09	0.02	<.0001	0.11	0.02	<.0001	0.13	0.03	<.0001	0.13	0.03	<.0001	0.13	0.02	<.0001	0.03	0.02	0.1
	Monocytes (×10E3/uL) *	0.08	0.02	0.001	0.11	0.03	<.0001	0.10	0.04	0.006	0.13	0.04	0.001	0.13	0.03	0.0001	0.04	0.03	0.08
	Eosinophils (×10E3/uL) *§	0.06	0.04	0.13	0.10	0.05	0.03	0.10	0.07	0.12	0.10	0.07	0.14	0.10	0.06	0.08	0.04	0.04	0.3

All models adjusted for sex (male, female), age in years (<35,35–50,>50), race (White, Black, Other), Body Mass Index (<24.9,25.0–29.9,>30), alcohol consumption (not current drinker, fewer than 7 drinks per week, more than 7 drinks per week), education (Less than high school or equivalent, High school diploma or GED, Some college or 2 year degree, 4 year college graduate), and household annual income (<$20000,$20001–$50000,>$50000).

*
ln-transformed.

**
Tobacco smoke-unexposed participants reported no primary tobacco use, less than 30 minutes of passive smoke exposure, and had blood 2,5-DMF concentrations less than 14 ng/L.

***
Tobacco smoke-exposed participants either reported primary tobacco use, 30 or more minutes of passive smoke exposure, or had blood 2,5-DMF concentrations greater than or equal to 14 ng/L..

§
Participants with eosinophil percentrage equal to zero were assigned a value of 0.5% to permit ln-transform.