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Peripheral blood effects in benzene-exposed workers

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ABSTRACT

The hematotoxic effects of benzene exposure may be important in the occurrence of subsequent health effects. We sought to provide further information on peripheral blood effects by studying 928 workers in five factories in and around Shanghai, China exposed to a wide range of benzene concentrations. Specifically, we sought to investigate which blood indices are more strongly related to benzene exposure and which concentration levels of benzene result in peripheral blood changes. Lifestyle habits and demographic information was obtained via questionnaire, and potentially important genetic influences were determined by assessing single nucleotide polymorphisms in four genes (NQO1, MPO, CYP2E1, GSTT1). Weekly benzene exposure estimated from individual monitoring results ranged from 0.07 to 872 mg/m^3 with a median value of 7.4 mg/m^3. Twelve peripheral blood indices were examined. Stronger effects on peripheral blood were seen for red cell indices such as anemia and macrocytosis, albeit at higher (>10 ppm) exposure levels. The most sensitive parameters to benzene appeared to be neutrophils and the mean platelet volume (MPV), where effects were seen for benzene air concentrations of 7.8–8.2 ppm. Toluene exposure is a potential confounder for some peripheral blood effects, pointing to the need to scrutinize levels of both compounds in the occupational environment.

1. Introduction

Benzene's effect on blood parameters has been studied for some time, with well-documented effects on erythrocytes, leukocytes and platelets [1–3], but there is still uncertainty regarding whether certain blood elements are more sensitive to benzene. Various investigators have suggested that the most sensitive blood parameter for benzene's effect is neutropenia [4], lymphocytopenia [5], anemia and related red cell indices such as macrocytosis [6–8] or earlier progenitor cells [9], with apparently no consensus. Alternatively, benzene may have a non-specific effect on the peripheral blood, if it targets very early blood forming elements in the bone marrow that are not yet committed to a particular cell line, or the bone marrow stromal cells, which are important in normal hematopoiesis regulation [10].

There is conflicting data on the importance of gene polymorphisms encoding for key metabolizing and detoxification enzymes on peripheral blood counts [11,12]. It is also not conclusive whether other factors, such as concomitant exposures and lifestyle habits, modulate benzene's effects. Toluene exposure has been shown to inhibit metabolism and subsequent myelotoxicity [13], albeit at relatively high concentrations. More recently, lower concentrations of toluene have been suggested to enhance benzene's effects on micronucleus induction [14]. Although several studies have examined the role of benzene exposure on peripheral blood indices, important questions remain, such as the shape of the dose–response relationship for different blood indices and whether

Abbreviations: AIC, Akaike's Information Criterion; BZ, benzene; BMI, body mass index; CI, confidence interval; CYP2E1, Cytochrome P450 2e1; GSTT1, glutathione-S-transferase; MCV, mean corpuscular volume; MPO, myeloperoxidase; MPV, mean platelet volume; NQO1, NAD(P)H quinine oxireductase; PLT, platelets; RBCs, red blood cells; RDW, red cell distribution width; SEG, similar exposure group; WBCs, white blood cells.

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a most sensitive indicator of hematopoiesis disruption can be identified.

In the present study, we sought to provide more understanding of the relationship between benzene exposure and peripheral blood counts. Specifically, we addressed two primary aims: (1) examine the quantitative relationship between benzene exposure and twelve specific peripheral blood indices, and (2) identify the most sensitive indicator of benzene exposure among the twelve measured peripheral blood indices.

2. Materials and methods

To address our study aims, we conducted a cross-sectional study of workers from five factories in and around Shanghai, China that used benzene in their production processes. Trained interviewers administered an in-person questionnaire to consenting benzene-exposed and -unexposed workers, who provided a sample of blood after the interview was completed. Individual-level benzene and toluene exposure measurements were obtained from a random sample of workers. Linear and logistic regression models were used to examine the relationship of benzene exposure and peripheral blood indices, while adjusting for the effects of potential confounders.

2.1. Facility description

Factory A is a rubber factory established in 1954. One workshop that manufactured hoses and belts was selected. Benzene was used as an adhesive solvent, glue application and as a softener for application of reinforcing materials. Non-exposed clerical workers in a separate building were also included. The rubber factory was visited in 2003, 2006, and 2007, with 103 workers represented in two visits and 23 workers represented in all three visits. For this paper, we treated each visit as a separate observation, since effects under study are expected to be relatively transient, and the visits were separated by more than a year.

Factory B, which manufactures shoes, was established in 1988. Workers from three separate buildings were included in the present study. One building used older technology where benzene-based glues were used to join the upper parts of shoes with soles. Shoes are also packaged and shipped in a section with lower benzene exposures. A second building had similar processes, but with lower benzene exposure due to better ventilation and newer technology. A third building was the source of unexposed workers involved in cutting fabrics for shoes that were ultimately assembled in the first and second buildings.

Factory C started as an insulation materials factory in 1973. Benzene has been used since the early 1980s, when the factory started making oil-resistant rubber planks for vessel sealing purposes. Benzene is used to aid soaking and mixing processes, and as a diluent for mixed ingredients on a heated roller/extruder. Besides benzene, other exposures could include sulphur, asbestos, and industrial gasoline.

Factory D is a pharmaceutical factory established in 1961 that began making intermediate products using benzene in 1999. Two workshops that used benzene were studied, one where exposure largely occurred via materials transfer (e.g. pouring raw materials into reactors), and a second where exposure occurred via recycled solvent containing 83% benzene. Unexposed workers from security, an aluminium chloride workshop, and maintenance in non-benzene areas were also recruited.

Factory E, which is a sister (rubber) factory to factory A, was established in rural Shanghai in 1998. Rubber hoses are manufactured, and processes are very similar to factory A, although newer equipment is in place.

2.2. Subject selection

All workers in the selected workshops above were eligible for the study. Between September 2003 and June 2007, several visits were made to each factory, with no follow-up of subjects after a factory visit. Workers were included in the final sample if they completed a consent form and questionnaire, and provided a blood sample. Participation rates were estimated to be between 95% and 99% and did not vary by factory. The study was approved by the Institutional Review Boards at the University of Colorado and Fudan University.

2.3. Study questionnaire (demographic and lifestyle information)

A questionnaire was administered in-person to each study subject, who was queried on a broad range of topics, including birth date, tobacco and alcohol use, height, weight, hobbies, medication use, existing diseases, and exposure to livestock and/or pesticides. Body mass index (BMI) was calculated from information on height and weight. Current use of tobacco and/or alcohol was scrutinized by comparing questionnaire responses on different aspects of usage; "current use" was sometimes inferred by responses to "ever use". Urinary cotinine levels were used to validate responses to smoking questions.

2.4. DNA isolation and storage

Genomic DNA was isolated from blood using a Qiagen QIAamp DNA isolation kit (Qiagen, Chatsworth, CA) according to the manufacturer's directions. Proteinase K was added to EDTA-treated peripheral blood, followed by the addition of Qiagen lysis buffer. After lysis at 70 °C, ethanol was added and the resulting solution was passed over an appropriately sized QIAamp DNA isolation column and spun. The columns were then washed with wash buffers, and the DNA was eluted with distilled water. A spectrophotometer was used to measure the absorbance of the DNA-containing eluate at 260 and 280 nm, and the concentration and purity of the DNA was recorded. Several aliquots of DNA were prepared and stored at −80 °C for future use.

2.5. Single nucleotide polymorphism (SNP) analysis/classification

Five SNP's involved in benzene metabolism (NQO1609C > T, NQO1465C > T, CYP2E11019C > T, MPO463G > A, and GSTT1 null) were determined by restriction fragment length polymorphism (RFLP) analysis. A list of genes studied, the primers and restriction enzymes used is included below. For each RFLP method, several positive and negative samples were subjected to DNA sequencing to verify the accuracy of the method.

Gene	Primers	Polymorphism/result
NQO1*2	F: 5′-TCCTCAGAGTGGCATTCTGC-3′ R: 5′-TCTCCTCATCCTGTACCTCT-3′	C609T Gain of *Hin*fI site
NQO1*3	F: 5′-TCAAGTTGGCTGACCAAGGACA-3′ R: 5′-CCTGCATCAGTACAGACCAC-3′	C465T Gain of *Msp*I site
MPO	F: 5′-GGTATAGGCACACAATGGTGAG-3′ R: 5′-GCAATGGTTCAAGCGATTCTTC-3′	G463A Loss of *Aci*I site
CYP2E1*5	F: 5′-CCAGTCGAGTCTACATTGTCA-3′ R: 5′-TTCATTCTGTCTTCTAACTGG-3′	C1019T Loss of *Rsa*I site
GSTT1	F: 5′-TTCCTTACTGGTCCTCACATCTC-3′ R: 5′-TCACCGGATCATGGCCAGCA-3′	Null genotype No PCR product
β-Globin	F: 5′-CAACTTCATCCACGTTCACC-3′ R: 5′-GAAGAGCCAAGGACAGGTAC-3′	*GSTT1* control

We examined the effect of SNP's using two different classification methods. First, we entered each SNP as categorical variables

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into regression models. We also examined specific combinations of SNP's expected to have similar effects. To this end we defined three additional variables (labelled MPO-NQO, Cyp-GST, and AllSNP in tables) based on genotype combinations. First, the MPO wild-type (GG) and NQO1*2 mutant (TT) combination is unfavourable, as it predicts lower levels of hydroquinone and catechol and higher levels of benzoquinones, which are more toxic. A favourable MPO-NQO1 sequence was defined as the presence of MPO AA or AG and NQO1*2 CC or CT alleles, with neutral outcome otherwise. Next, the CYP2E1 CC and GSTT1 null genotype combination was also defined as unfavourable, due to their roles in activation and detoxification, respectively. The favourable sequence involved GSTT1 positive and CYP2E1 CT or TT alleles, with neutral defined otherwise. Finally, we looked at all five SNP's and defined an unfavourable combination as the presence of three or more unfavourable genotypes (viz. MPO GG, NQO1*2 TT, NQO1*3 TT, CYP2E1 CC, and GSTT1 negative), a favourable combination as the presence of four or more favourable genotypes, with no more than one unfavourable genotype, and neutral otherwise.

2.6. Peripheral blood indices

The present study used twelve peripheral blood indices derived from a complete blood count (CBC) test. Peripheral blood was collected by venipuncture from all individuals and processed for routine CBC using an automated hematology analyzer (CellDyne 3700, Abbott Park, IL). The twelve indices were selected based on biologic plausibility as well as previous literature on benzene. The indices were: total white blood cells (WBCs), five WBC subtypes (viz. neutrophils, basophils, eosinophils, monocytes, and lymphocytes), total red blood cells (RBCs), three red cell-related measures (viz. hemoglobin, mean corpuscular volume (MCV), and red cell distribution width (RDW)), platelets, and mean platelet volume (MPV). National normal ranges applicable to the Chinese population were obtained from the Joint Clinical and Molecular Laboratory in Shanghai for each parameter. Abnormal low and high blood readings were defined based on these ranges, to derive a more clinically relevant outcome. We defined abnormal high values for MCV, or macrocytosis, and abnormal low values for ten other indices, while no normal ranges were obtained for RDW.

2.7. Exposure assessment

To measure benzene exposure, we assigned workers ($n = 1046$) from each of the five study factories to one of 133 similar exposure groups (SEGs), which were defined as groups of workers with similar job, locations, assignments, tasks, work patterns, schedules and materials used. A random sample of workers was then selected from each SEG to obtain individual-level measurements. Monitored workers wore 3M® organic vapor badges on their lapel for 1–16 h during their work shift. We obtained a total of 2973 benzene samples with an average of four samples (range: 1–14) per worker. Badges were sealed, then transported to and analyzed at the Fudan University School of Public Health Analytical Laboratory following NIOSH Methods 1501 and 4000. Benzene and toluene showed limits of detection (LOD's) of 0.1 and 0.16 mg/m^3, respectively. Forty-four workers wore an additional badge on their opposite lapel to produce duplicate samples for benzene that were sent to an independent laboratory for analysis of agreement. Technicians were blinded to the result from the corresponding laboratory.

For each monitored worker in an SEG, we computed the time-weighted arithmetic mean of their individual-level benzene and toluene measurements. For each SEG, we also computed the arithmetic mean of the time-weighted averages for benzene and toluene and assigned the resulting measurement value to each individual within an SEG. The benzene exposure index that was ultimately used in this paper (see the statistical analysis section for rationale) was based on individual weekly average readings and imputed values from the SEG weekly average reading if the latter was from a homogeneous SEG (see Fig. 1). We defined homogeneous SEG's ($n = 88$, 66% of SEG's) as having a ratio of the upper and lower 95% confidence limits for the between worker variance of 4 or less as determined by a random effects ANOVA model. We compared exposure distributions for homogeneous vs. all other SEGs, with no patterns to suggest that a bias would result by restricting exposure data to sufficiently homogenous SEG's.

2.8. Statistical analysis

To examine the association between benzene exposure and the twelve peripheral blood parameters, we initially examined correlations and simple regressions between blood parameters and three benzene indices (i.e. (a) SEG weekly averages, (b) individual exposure weekly averages, and (c) individual weekly averages with imputed values from SEG averages of homogeneous SEG's). The three metrics were highly correlated (range 0.70–0.90) and crude associations were robust to varying metrics of exposure assessment. Therefore, we chose to estimate benzene exposure using individual-level weekly averages with imputed averages from sufficiently homogenous SEGs for individuals who had not been monitored, as the method is based on individual readings, accounts for potential heterogeneity within an SEG, and is subject to a relatively small loss in power. We transformed exposure levels at or below the LOD for benzene (0.10), and toluene (0.16) with the formula LOD/$\sqrt{2}$. Exposure distributions were right-skewed and

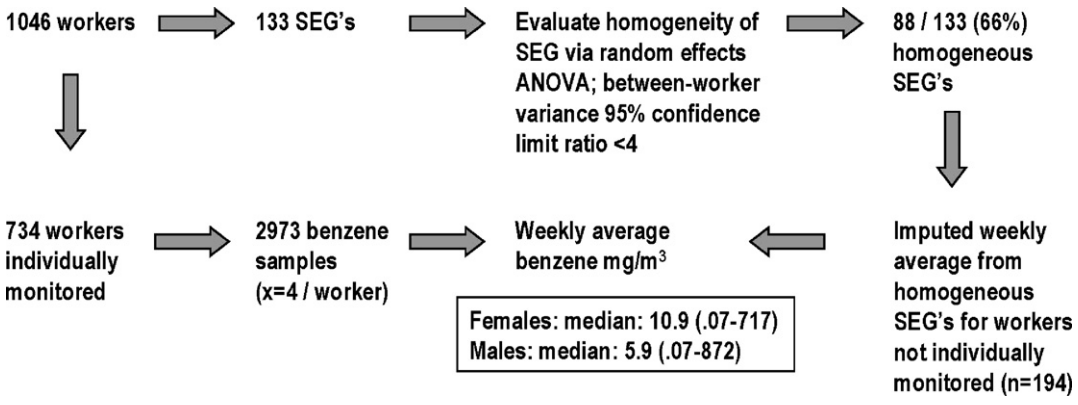

Fig. 1. Exposure assessment process for study subjects.

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Table 1
Characteristics of study population[a].

	Total population (n = 1046)		Subjects with exposure index (n = 928)	
	Female	Male	Female	Male
	N (%)	N (%)	N (%)	N (%)
Factory A	102 (9.8)	250 (23.9)	101 (10.9)	240 (25.9)
Factory B	299 (28.6)	113 (10.8)	246 (26.5)	93 (10.0)
Factory C	58 (5.5)	68 (6.5)	49 (5.3)	60 (6.5)
Factory D	12 (1.2)	116 (11.1)	11 (1.2)	110 (11.9)
Factory E	12 (1.2)	16 (1.5)	9 (1.0)	9 (1.0)
Age (median)	34.6	42.6	34.9	42.6
Benzene exposure (median, range) (mg/m^3)	–	–	10.9 (0.07–717)	5.9 (0.07–872)
Non-smokers	468 (44.7)	188 (18.0)	401 (43.2)	162 (17.5)
Smokers	11 (1.1)	372 (35.6)	11 (1.2)	348 (37.5)
No Alc. use	467 (44.7)	238 (22.8)	405 (43.6)	208 (22.4)
Alcohol use	16 (1.5)	325 (31.1)	11 (1.2)	304 (32.8)
Body mass index (median)	21.2	22.4	21.2	22.4
CYP2E1 C1C1	283 (27.1)	349 (33.4)	247 (26.6)	319 (34.4)
C1C2	172 (16.4)	192 (18.4)	145 (15.6)	175 (18.9)
C2C2	28 (2.7)	22 (2.1)	24 (2.6)	18 (2.0)
MPO A/A	10 (1.0)	7 (0.7)	8 (0.9)	5 (0.5)
A/G	130 (12.4)	132 (12.6)	114 (12.3)	119 (12.8)
G/G	343 (32.8)	424 (40.5)	294 (31.7)	388 (41.8)
NQO1*2 C/C	141 (13.5)	175 (16.7)	120 (12.9)	156 (16.8)
C/T	252 (24.1)	267 (25.5)	221 (23.8)	244 (26.3)
T/T	90 (8.6)	121 (11.6)	75 (8.1)	112 (12.1)
NQO1*3 C/C	460 (44.0)	527 (50.4)	399 (43.0)	481 (51.8)
C/T or T/T	23 (2.2)	36 (3.5)	17 (1.8)	31 (3.3)
GSTT1 negative	236 (22.6)	289 (27.6)	200 (21.6)	265 (28.6)
Positive	247 (23.6)	274 (26.2)	216 (23.3)	247 (26.6)

[a] Numbers do not always add due to missing values.

we applied the natural logarithm to reduce undue influence that outlying observations could have on model-estimated parameters.

We used generalized linear models (GLM) to evaluate the associations between benzene exposure and peripheral blood parameters. We followed an iterative process of model evaluation and selection to identify important confounders in the exposure/outcome relation whereby each candidate covariate was sequentially assessed by comparing the Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) of nested models fitted with benzene exposure. A p-value of 0.20 was chosen to evaluate entry and exit into models, as this value more readily identifies important confounders by change-of-coefficient (COC) criteria [15]. Parsimony and change in the benzene coefficient were also considered in model development. After exploring several variables in simple regressions, and considering potential power loss for missing covariates, candidate confounders of the benzene/blood parameter relationships included worker age, gender, body mass index (BMI), current smoking status (yes/no), current alcohol use (yes/no), SNP's and SNP combinations, and co-exposure to toluene. SNP data were treated as categorical values with either the wild-type (for original SNP's) or favourable genotype (for SNP combinations) entered as baseline. In addition, to evaluate clinically relevant endpoints, we fit logistic models with blood parameters using high or low out-of-range values as described previously. We also conducted change point regressions to identify benzene concentrations that produce blood count changes distinguishable from background levels for each parameter.

All statistical analyses were performed using version 9.2 in SAS (SAS Cary, NC).

3. Results

3.1. Description of study group

Of the original 1078 workers who participated in the study, 32 subjects were excluded from the final analysis because they were not currently working (n = 20) or had hepatitis (n = 10), a pre-existing blood disorder (n = 1), or a blood transfusion in the prior six months (n = 1). This resulted in 1046 workers eligible for analyses. Two additional workers who did not have valid blood counts were excluded from the study. All 1046 workers were assigned to a SEG. The benzene exposure index used in analysis was defined for 928 of the 1046 workers, and was based on either individual weekly average readings (n = 734) or the SEG weekly average if it was from a homogeneous SEG (n = 194).

Demographic characteristics and other potential confounders are presented in Table 1 by gender for the overall study population and subjects with individual benzene measurements (n = 928). Workers were 54% male vs. 46% female. Males were older than females (medians, 42.6 years vs. 34.5 years) and much more likely to report being a current smoker (35% vs. 1%) or alcohol drinker (31% vs. 2%). Female workers had about twice the average benzene concentration measurements than their male counterparts (10.9 mg/m^3 vs. 5.9 mg/m^3). Among the 928 study subjects with individual benzene measurements, distributions of covariates by age were nearly identical to those of the total study sample.

Inter-laboratory agreement of duplicate benzene air monitoring results is displayed in Fig. 2. Disagreements between the laboratories were small; the correlation coefficient for replicate samples is 0.99. Benzene exposure was widely distributed (range = LOD/$\sqrt{2}$ = 0.07 mg/m^3 (0.02 ppm) to 872 mg/m^3 (273 ppm)) but right-skewed. Interquartile values were 0.9 mg/m^3 (0.3 ppm), 7.4 mg/m^3 (2.3 ppm), and 29.5 mg/m^3 (9.2 ppm). Of the 928 workers, 73 were controls who worked at the same factory, but were not exposed to benzene. Exposed and control counts by factory are as follows: Factory A: 328/13; Factory B: 321/18; Factory C: 108/1; Factory D: 80/41; and Factory E: 18/0.

3.2. Individual benzene exposure and blood parameters

Most peripheral blood parameters were normally distributed, except for eosinophils, which were skewed right, necessitating a

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Table 2
Results of simple and multivariable generalized linear regression models of benzene exposure by blood parameter.

Blood parameter	n	Crude β	Crude R^2	Adjusted β	Adjusted R^2	Covariates controlled for
WBCs	909	−0.06[a]	0.01	−0.06[b]	0.06	Smoking, age, BMI
Lymphocytes	909	−0.01	0.00	−0.02[a]	0.15	Age, BMI
Monocytes	909	−0.00	0.01	−0.00[a]	0.03	Smoking, age, BMI, GSTT1, NQO1*3
Neutrophils	909	−0.05[b]	0.01	−0.01[a]	0.05	Smoking, age, BMI, NQO1*2, AllSNP
Eosinophils[d]	908	0.01	0.00	0.01	0.01	Smoking, age, BMI, CYP2E1, MPO-NQO
Basophils	909	−0.00[a]	0.00	−0.00	0.03	Age, MPO-NQO
RBCs	908	−0.06[c]	0.05	−0.04[c]	0.45	Smoking, gender, age, alcohol, BMI, Cyp-GST
Hemoglobin	908	−0.14[a]	0.03	−0.07[c]	0.60	Smoking, gender, age, BMI, GSTT1, NQO1*2, Cyp-GST
MCV	908	0.26[c]	0.01	0.36[c]	0.11	Gender, age, alcohol
RDW	907	0.03	0.00	0.02	0.03	Gender, Cyp-GST
Platelets	908	−1.64	0.00	−2.32[b]	0.07	Gender, age, CYP2E1, NQO1*2
MPV	840	−0.18[c]	0.03	−0.18[c]	0.04	Gender, age, alcohol, BMI

[a] $p < .05$.
[b] $p < .01$.
[c] $p < .001$.
[d] Log transformed.

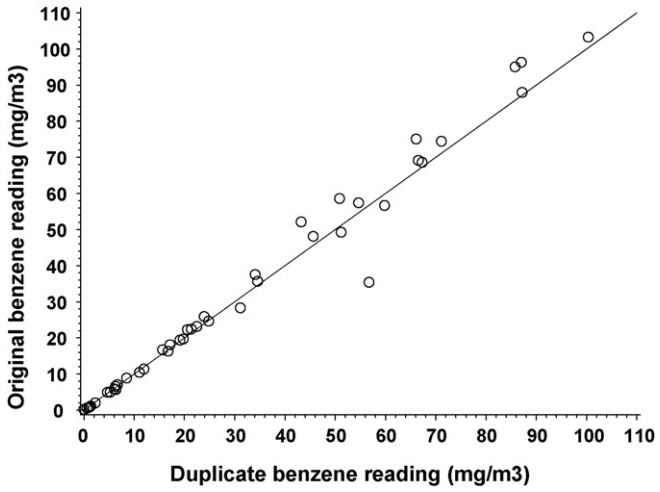

Fig. 2. Scatter plot of replicate benzene air measurements from two laboratories.

logarithmic transformation to satisfy regression model requirements. The proportion of workers whose blood parameter values fell outside the range of clinically normal limits was generally small, with a maximum of fewer than 8% for hemoglobin. There were too few workers with abnormally low readings of monocytes ($n = 1$), MPV ($n = 1$) or basophils ($n = 0$) for meaningful statistical analyses via logistic regression, while no clinical norms were available for RDW.

Table 2 presents crude and adjusted beta coefficients of the relationship between logged benzene exposure and each of the twelve blood parameters, adjusted for potential covariates. Among the three types of blood elements (WBCs, RBCs and platelets), stronger patterns of association emerged for total RBCs and its constituents. Specifically, we observed statistically significant decrements of 0.04 ($p < .001$) for RBC count and 0.07 ($p < .001$) for hemoglobin, with a 0.36 ($p < .001$) increase in MCV for a one-unit increase in the log of individual-level benzene concentration. We also observed small, but clear signals for total platelet count and MPV, with reductions of 2.32 ($p < .01$), and 0.18 ($p < .001$) per log of benzene concentration. In general, the reductions in WBC parameters were attenuated and less significant than those noted above. Among this cell lineage, we observed the greatest magnitude of effect for total WBC counts ($\beta = -0.06$; $p < .01$). Weaker signals were seen with WBC differentials, although lymphocytes and neutrophils, two subtypes previously hypothesized to be more sensitive to benzene exposure, both showed small, statistically significant effects (lymphocytes: $\beta = -0.02$; $p < .05$; neutrophils: $\beta = -0.01$; $p < .05$).

The results summarized above are pertinent when toluene is not eligible for model inclusion. However, when we included a measure of continuous toluene exposure as a term eligible for models, we observed a marked reduction on the benzene effect for some blood cell elements. Specifically, toluene reduced the benzene effect to non-significance for the following outcomes: neutrophils, monocytes, RBC's, haemoglobin, MCV, and platelets. Toluene enhanced the inverse benzene effect on lymphocytes, and had no effect on the benzene coefficient for WBC's and MPV (which remained significant), and basophils and RDW (which were not influenced by benzene). Toluene and benzene show a moderately strong correlation ($r = 0.66$), and some of the effect is likely due to this collinearity. We are further researching the effect of toluene exposure on blood parameters in this population.

Results for logistic regression models that examined out-of-range values generally supported the continuous models (Table 3) in that stronger effects were seen for red cell parameters, although fewer blood parameters were related to benzene. There were 55 workers who were anemic, showing RBC values lower than normal ranges, and benzene exposure showed a statistically significant

Table 3
Results of logistic regression models for out-of-normal range blood indices.

Outcome	N	OR	95% CI	Covariates	AIC
WBC	32	1.18	1.00, 1.39	Age, BMI, smoking, gender	261.4
Lymphocytes	46	1.04	0.91, 1.19	Age	310.92
Neutrophils	46	1.05	0.92, 1.20	Smoking, BMI, age	856.4
Eosinophils	21	1.07	0.88, 1.30	Cyp-GST, MPO, BMI	195.4
RBC	55	1.28	1.12, 1.47	Age, MPO-NQO, BMI, gender	388.5
MCV	31	1.68	1.35, 2.10	–	246.3
Hemoglobin	69	1.11	0.99, 1.24	Gender, age, GSTT1, smoking, MPO-NQO	459.0
Platelets	16	1.28	1.01, 1.63	Age, BMI	158.2

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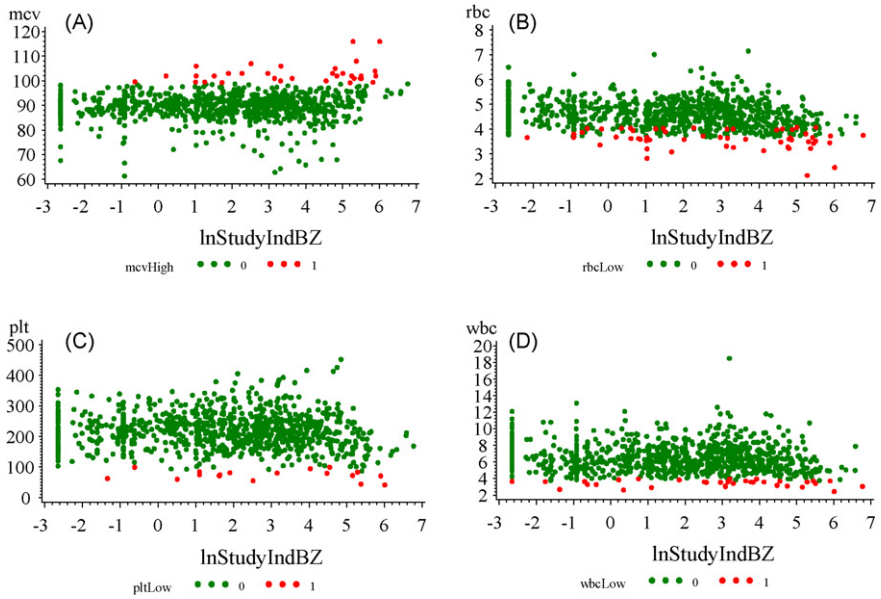

Fig. 3. Out-of-range and normal blood values by benzene exposure.

odds ratio (OR = 1.28, 95% CI = 1.12–1.47) effect for this outcome, after controlling for effects of age, MPO/NQO1 alleles, BMI and gender. Thirty-one workers were defined as having macrocytosis by showing high MCV values. As in continuous models, this measure showed the strongest relationships with benzene (OR = 1.68, 95% CI = 1.35–2.10). No covariates confounded this relationship. There were 69 workers who had an abnormally low reading for haemoglobin, and benzene exposure showed an OR of 1.11 (95% CI = 0.99–1.24) for this outcome. Only 16 workers were defined as having thrombocytopenia by their low platelet readings, although logistic models showed a significant OR of 1.28 (95% CI = 1.01–1.63) for an increment of log benzene and this outcome.

As with continuous models, WBC and related parameters showed weaker results for the out-of-range outcomes compared to red cell and platelet indices. The only outcome related to benzene was leukopenia where benzene exposure had a small (OR = 1.18), but statistically significant (95% CI = 1.00–1.39) effect. On the other hand, lymphocytopenia, neutropenia, and eosinopenia were not significantly related to benzene exposure (OR's = 1.04, 1.05, and 1.07 respectively). Thus, the clinically relevant blood indices most affected by benzene were, in order of strength: macrocytosis, anemia, thrombocytopenia, and leukopenia. Fig. 3a–d displays the out-of-range values for these four endpoints, respectively, by benzene exposure. Fig. 3a shows a high percentage of out-of-range MCV values for higher benzene exposures, supporting the relatively larger OR for macrocytosis from the regression modelling results. Fig. 3b–d shows progressively smaller fractions of out-of-range values for the other three parameters, again supporting the modelling results for each corresponding endpoint.

In order to more formally assess the benzene concentrations that affect each blood parameter, we fit change point regression models to each parameter that had a significant effect in general linear models. We generally did not include toluene exposure in these models. The lowest change point is a candidate for the most sensitive effect due to benzene exposure. These results are displayed in Table 4. Neutrophil counts and the MPV had the lowest change points of about 8 ppm. Notably, lymphocyte counts showed the highest change point, in excess of 30 ppm. Inclusion of toluene in this model had a negligible affect on the lymphocyte change point.

To further assess dose response for the out-of-range indices that showed effects in logistic regressions, we categorized benzene exposures as <1 ppm, 1–<10 ppm, and 10+ ppm. Results are summarized in Table 5. Stronger effects were seen for MCV and RBC, with highly significant OR's in the >10 ppm exposure category. MCV shows a monotonic risk, while RBC's show an irregular dose–response, making it more difficult to interpret.

4. Discussion

Our study showed that a wide range of benzene exposures affect most, but not all, peripheral blood cell indices. When blood indices were measured continuously, we report effects for aggregate measures of the three cell lineages (viz. RBC, WBC, and PLT), two of three other red cell measures (HGB, MCV), three of five other WBC parameters (lymphocytes, neutrophils and monocytes), as well as

Table 4
Change point concentrations for selected blood parameters.

Blood parameter	Change-point (log BZ mg/m^3, BZ ppm)	95% CI for log BZ (mg/m^3)	Slope	95% CI	Toluene term
WBC	4.18, 20.5	3.46, 4.90	−0.68	−1.19, −0.17	No
Lymphocytes	4.80, 38.1	4.14, 5.47	−0.41	−0.80, −0.01	No
Lymphocytes	4.78, 37.3	4.10, 5.46	−0.45	−0.85, −0.04	Yes
Neutrophils	3.21, 7.77	2.25, 4.16	−0.29	−0.48, −0.09	No
RBC	3.70, 12.7	3.21, 4.19	−0.23	−0.33, −0.14	No
Hemoglobin	3.43, 9.68	2.65, 4.21	−0.35	−0.56, −0.14	No
MCV	4.47, 27.4	4.12, 4.82	4.96	2.90, 7.02	No
Platelets	3.62, 11.7	2.97, 4.28	−21.7	−33.3, −10.2	No
MPV	3.27, 8.24	2.04, 4.52	−0.42	−0.80	No

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Table 5
Odds ratios[a] by benzene exposure category for significant clinically relevant outcomes.

Blood parameter	<1 ppm, 95% CI	1–<10 ppm, 95% CI	10+ ppm, 95% CI
WBC	2.49 0.31, 20.0	1.92 0.23, 15.7	4.07 0.51, 32.4
RBC	10.8 1.41, 82.5	5.13 0.66, 39.9	16.0 2.11, 121
MCV	5.65 0.63, 51.1	5.91 0.75, 46.5	17.7 2.35, 134.1
PLT	2.18 0.24, 19.8	1.76 0.20, 15.2	4.54 0.56, 36.7

[a] Baseline categories were defined as no-exposure (i.e. <0.071 mg/m^3 benzene), unless the baseline category contained no workers. For RBC and PLT, baseline was <0.32 mg/m^3 and for MCV, baseline was <0.54 mg/m^3.

MPV. Toluene exposure may be a confounder for many of these reported relationships. We also examined peripheral blood effects in a more clinically relevant manner, again observing effects for the three aggregate cell lineages, but also seeing the strongest effect for macrocytosis, albeit at higher concentrations.

There are several strengths present in this study. The study involved a wide range of exposures, enabling us to distinguish concentrations of benzene which had effects. Over 2900 benzene measurements were taken for the study so that fairly precise measures of exposure could be developed for individual workers. We accounted for potential heterogeneity in readings by using worker time-weighted average exposures. While we supplemented individually monitored workers with workers where SEG readings were available, we ensured that the SEG's were reasonably homogeneous before using this data. The study also involved a large number of workers; to our knowledge it may be the largest study where both individual benzene readings and blood counts were analyzed. This enhances the ability to pick up subtle effects and guards against false positive findings. In addition, we were able to account for several other possible influences on blood values by collecting information on SNP's for key genes, other chemical exposures, demographics, lifestyle habits as well as other medical information.

Our study should also be evaluated in light of its weaknesses. First, by using a cross-sectional design, we were unable to directly account for historical effects on blood parameters. Repeated blood count measures would have aided the assessment of the clinical severity of the blood findings to provide added insight on whether the blood effects might be precursors to more severe effects. Second, the five factories produced different products with potentially different co-exposures, e.g. exposure to a greater variety of chemicals was present in the sealant products and pharmaceutical factories, though these were not thought to affect the blood. Finally, the genetic variants we included were not all-encompassing, and it is likely that other genetic variations do play a role in benzene toxicity [16].

The fact that we found benzene effects on several blood parameters is not surprising, since several other studies have also observed effects on multiple cell lineages [4,9] and benzene is a known cause of pancytopenia [17]. Our finding of possibly stronger effects for erythrocytes (and related parameters, particularly MCV) rather than leukocytes is both consistent [7,18,19] and inconsistent [5,20,21] with previous literature. Precise prediction of the lineage that is most strongly related to benzene may depend on more precise measurement of other known influences on blood parameters, such as the level of alcohol use or other co-exposures over a prescribed time period.

Our finding that neutrophils displayed the lowest change point in regression analyses suggests that it may be the more sensitive indicator of benzene's effects. There is some precedent for this observation, as Qu et al. [4] also suggested that neutrophils were the most sensitive parameter when examining several indices against a categorical grouping of benzene exposure. However, Qu et al. [22] reported that the group with lowest benzene exposure (<0.5 ppm) showed decreased neutrophils, while our data suggests that levels of approximately 8 ppm are necessary to distinguish neutrophil readings from background variation. Qu et al. [22] also reported decreased neutrophil counts among workers with lower cumulative exposure and found that the median exposure was 2.7 ppm with 14 years of exposure. Thus, higher past levels of exposure may have influenced neutrophil counts.

Other investigators have suggested that lymphocytes are the most sensitive effect of benzene exposure [5,21]. Rothman et al. [5] found decreased lymphocyte counts in a subgroup of workers exposed to a median level of 7.6 ppm, while Lan et al. [9] observed similar levels in groups exposed to <1 ppm and 1–<10 ppm (both of which differed from controls), and a clearer reduction for those exposed >10 ppm. Interestingly, Qu et al. [22] who also looked at categories of exposure reported lymphocyte reductions only in the highest exposed subgroup (>30 ppm), and Ward et al. [20] did not report evidence of lymphocyte reductions in rubber hydrochloride workers. Our data also suggest an effect for lymphocytes for higher (viz., 38 ppm) exposures. We were able to find only one other recent study on MPV [12], but no effect was found. While there is little experience with the MPV parameter, it is possible that it is an earlier indicator of platelet effects. However, our findings suggesting that this parameter may be a sensitive indicator of a benzene effect awaits further confirmation.

When we examined more clinically relevant outcome measures for blood indices based on normal ranges established for the Chinese population, we found effects for macrocytosis, anemia, thrombocytopenia and leukopenia, with dose-related effects that were clearer for macrocytosis and anemia. While anemia showed an effect in logistic models for <1 ppm, this may or may not be a real effect since the 1–10 ppm grouping did not show a clear effect. Collins et al. [23] used this strategy but did not find lymphocytopenia for low benzene exposures (1 ppm), while Ward et al. [20] found leukopenia for 10 ppm benzene exposures.

One aspect of our findings that should not be overlooked is that the concentrations that were associated with most blood effects (i.e. >7 ppm) are higher than levels that have previously been associated with changes in metabolite levels [24] (e.g. 0.3 ppm). This suggests that benzene levels that result in measurable effects on metabolites do not necessarily correspond to effects on peripheral blood elements, which are probably closer to biomarkers of effect, especially when they are irreversible.

Toluene exposure was seen to significantly confound our findings on benzene exposure for many blood cell populations. The blood has not thought to have been a critical target tissue following toluene exposure [25]. Rosin et al. [26] reported effects on red cell parameters in rats including macrocytosis and erythrocyte counts when 12,000 mg/m^3 toluene was co-administered with ethanol, but no effect for toluene alone. Others have reported that workers exposed to mixed solvents, where toluene was prevalent, experience reduction in T lymphocytes, but increases in B lymphocytes [27,28]. However, Akbas et al. [29] report no effect on total lymphocytes or leukocytes in toluene-exposed workers. Previous studies [22,30] have adjusted for toluene exposure, and report little influence on metabolite levels or blood parameters. However, this adjustment was done via a dichotomization of toluene exposure, a technique subject to information loss and misclassification. Thus, there is rather sparse data on quantitative toluene exposures and the potential effect on the blood. We will be reporting more detailed results concerning toluene exposure in the future.

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In summary, this study has shown that absolute neutrophil counts as well as reduced MPV may be a sensitive blood parameter for benzene exposure. Higher benzene exposures are more strongly related to red cell parameters, including MCV. Effects on peripheral blood counts also suggest the importance of toluene exposure, suggesting that it is important to scrutinize the levels of both compounds in the workplace.

Conflict of interest

None. Employed by ExxonMobil Biomedical Sciences, Inc. Other ExxonMobil businesses manufacture or use substances that may contain benzene.

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