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Nonlinear low dose hematotoxicity of benzene; a pooled analyses of two studies among Chinese exposed workers

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ABSTRACT

Background: Impairment of the hematopoietic system is one of the primary adverse health effects from exposure to benzene. We previously have shown that exposure to benzene at low levels (<1 ppm) affects the blood forming system and that these effects were proportionally stronger at lower versus higher levels of benzene exposure. This observation is potentially explained by saturation of enzymatic systems.
Methods: Here we extend these analyses by detailed modeling of the exposure response association of benzene and its major metabolites (i.e. catechol, muconic acid, phenol, and hydroquinone) on peripheral white blood cell (WBC) counts and its major cell-subtypes (i.e. granulocytes, lymphocytes, and monocytes) using two previously published cross-sectional studies among occupationally exposed Chinese workers.
Results: Supra-linear exposure response associations were observed between air benzene concentrations (range ~ 0.1 – 100 ppm) and WBC counts and its cell-subtypes, with a larger than proportional decrease in cell counts at lower than at higher levels of benzene exposure. The hematotoxicity associations were largely similar in shape when the analyses were repeated with benzene urinary metabolites suggesting that enzymatic saturation is not a full explanation of the observed non-linearity with WBC endpoints.
Discussion: We hypothesize that the flattening of the exposure response curve especially at higher benzene exposure levels may reflect a response by the bone marrow to maintain hematopoietic homeostasis. Toxicity to the bone marrow and an induced hyper-proliferative response could both contribute to risk of subsequently developing a hematopoietic malignancy. Additional work is needed to explore this hypothesis.

1. Introduction

Benzene, an aromatic hydrocarbon, is and has been used in many industrial processes to manufacture chemicals (e.g. styrene, acetone), rubbers, lubricants, dyes, detergent, drugs, and pesticides. Benzene also occurs in the general environment as it is a natural constituent of crude oil and gasoline. Although, occupational and environmental concentrations have generally decreased over the last decades, concerns remain about the health effects of benzene at low concentrations.

An IARC working group in 2017 reaffirmed the conclusion that there is sufficient evidence in humans that benzene causes acute myeloid leukemia (Loomis et al., 2017). In addition, the working group cited positive associations with acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma and non-Hodgkin lymphoma. The observation that benzene is possibly related to several lymphohematopoietic cancers is supported by the fact that there is strong evidence that

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benzene metabolites induce multiple effects at the level of the hematopoietic stem cell (HSC) resulting in biological effects including hematotoxicity and chromosomal changes (Loomis et al., 2017; Lan et al., 2004; McHale et al., 2012).

Impairment of the hematopoietic system, including granulocytopenia, lymphocytopenia, pancytopenia, and aplastic anemia, is one of the primary (non-carcinogenic) adverse health effects of benzene (Aksoy et al., 1972). We and others have shown that benzene can cause hematological effects, as measured by decreases in peripheral blood cells, over a wide range of benzene exposure levels (Bauer et al., 2003; Pesatori et al., 2009; Qu et al., 2002; Rinsky et al., 2002; Rothman et al., 1996; Ward et al., 1996; Schnatter et al., 2020). Hematotoxic effects are hypothesized to contribute to leukemogenesis from benzene by affecting HSCs and by interacting with supportive stromal cells and mature lymphocytes (Baan et al., 2009; Morgan and Alvares, 2005). Hematological changes to the stromal microenvironment could allow for the clonal expansion of leukemic stem cells (Dewi et al., 2020). This mode of action for benzene fits with the known ability of benzene metabolites to induce chromosomal changes and genomic instability in blood cells and progenitor cells, and with the fact that benzene poisoning (defined as having a total white blood cell (WBC) count $< 4000/\mu l$ measured repeatedly over several months, a compensable condition in China), is associated with a more than 70-fold increased risk for hematopoietic malignancies later in life (Rothman et al., 1997).

Although the hazards of benzene induced hematological effects are generally accepted, there is continuing debate about the shape of the exposure–response curve (ERC) at low levels of benzene exposure including the existence of functional thresholds (Vlaanderen et al., 2010). We previously have shown that the association between benzene exposure and peripheral WBC counts and pluripotent stems cells was non-linear with a steeper slope at lower levels than at higher levels of benzene exposure (supra-linear). (Lan et al., 2004) A supra-linear ERC was also observed in benzene related transcriptomic response patterns in disease-relevant pathways (Thomas et al., 2014). In addition, a quantitative *meta*-regression on benzene exposure and leukemia risk in occupational cohort studies provided some support for a supra-linear association (Vlaanderen et al., 2010). These lines of evidence would point towards a proportional stronger increase in adverse effects at lower levels than at higher levels of benzene exposure. This observation is potentially important when extrapolating risks from studies performed at higher levels of exposure to low occupational and environmental concentrations.

We previously hypothesized that the supra-linear shape of the benzene-WBC and possibly the benzene-leukemia ERC is (in part) the result of enzymatic saturation of the pathways generating benzene's most toxic metabolites at higher levels of benzene exposure (Kim et al., 2006). A previous study indicated that the dose-related production of the major benzene metabolites (catechol (CA), hydroquinone (HQ), muconic acid (MA), and phenol (PH)) declines from complete proportionality to between 2.5 and 26-fold less production as benzene exposure increases. These reductions were most pronounced for CA and PH (Kim et al., 2006). Here we describe detailed pooled analyses of two cross-sectional studies among Chinese benzene exposed workers and hematotoxicity across a wide range of benzene exposure (~0.1 – 100 ppm) and explore the shape of the ERC based on individually measured air benzene concentrations and urinary benzene-metabolites CA, HQ, MA, and PH. If metabolic saturation would play a role in the previously observed supra-linear effects of air benzene on blood cell counts expectation would be that in the case urinary metabolites reflect (more) the concentration of toxic metabolites at the target organ that the obtained exposure–response-curves with urinary metabolites would be more linear. Here we test this hypothesis by exploring the relationship between a wide range of benzene exposure, the urinary benzene metabolites, and hematotoxicity.

2. Material and methods

2.1. Study populations

Data from two studies of Chinese workers exposed to benzene were used in this analysis. Details of the two studies have been reported previously. In brief, the study reported by Lan et al. (2004) was conducted in 2000/2001 in Tianjin, China (hereafter referred to as the NCI-study). Exposed workers (n = 250) were enrolled from two shoe manufacturing factories. Occupationally unexposed controls (n = 140), for ease referred to in this paper as 'unexposed', were selected from three clothes manufacturing factories that did not use benzene or other chemicals associated with bone marrow toxicity in the same geographical region. Controls were frequency-matched by sex and age to exposed workers. The study reported by Qu et al. (2002) was conducted in 2001 also in Tianjin, China (hereafter referred to as the NYU-study). Exposed workers (n = 130) were enrolled from three factories with broad ranges of benzene exposure; a glue factory, a shoe making factory, and a sporting goods company. Two nearby workplaces in the same geographic area that did not use benzene or chemicals associated with bone marrow toxicity were selected to enroll occupationally unexposed workers (n = 51), as for the NCI-study this group is referred to as 'unexposed': a food processing factory and a flour factory. Unexposed workers were frequency-matched on age, gender and smoking status to workers currently exposed to benzene. Subjects in both studies were administered a questionnaire requesting information on occupational history, environmental exposures, medical history and current medications, past and current tobacco and alcohol use, weight, and height. All subjects provided a peripheral blood sample.

2.2. Blood cell counts and differentiation

In the NCI study the complete blood count (CBC) was analyzed using a Beckman-Coulter® T540 blood counter, and the major lymphocyte subsets were analyzed by a Becton Dickinson FACS Calibur flow cytometer (Lan et al., 2004). In the NYU study WBCs were counted by a cell counter (Model PC603, Beijing China). The WBC differential was manually counted on a total of 900 cells by a commercial laboratory (Quest Diagnostics, San Diego, CA) (Qu et al., 2002). We did not include erythrocytes or platelets in the current analyses due to missing values and as these have previously shown to be not the most sensitive endpoints (Lan et al., 2004; Qu et al., 2002).

2.3. Exposure assessment

In the NCI study personal full-shift air monitoring using 3 M® organic vapor monitors (OVMs) took place every 1–2 months over a 16-month period in the larger factory which had lower benzene levels and 5 times in the factory with higher exposures. OVMs were analyzed for benzene, toluene and xylene by gas chromatography. Average benzene exposures were calculated by taking the arithmetic mean of the subject's individual measurements in the last month (2 to 3 measurements per individual). Further details of the exposure assessment approach in the NCI study have previously been described in detail (Vermeulen et al., 2004). In the NYU study individual exposure was also measured using 3 M® OVMs over a 4-week period before blood collection. Similar to the NCI study, average benzene exposures were calculated by taking the arithmetic mean of the subject's individual measurements in the last month (3 to 4 measurements per individual) (Qu et al., 2002).

In both studies, spot urine samples were collected at the end of the work shift. Urine samples were analyzed for benzene, catechol (CA), hydroquinone (HQ), muconic acid (MA), S-phenylmercapturic (SPMA) and phenol (PH) by gas chromatography-mass spectrometry in the same laboratory (Kim et al., 2006). We did not include SPMA in the current analyses as SPMA did not show a saturable pattern in previous analyses and therefore is not informative to the research question of metabolic

saturation can explain non-linear exposure response curves (Lan et al., 2004).

2.4. Statistical analysis

After exclusion of subjects with missing data on exposure, outcome, or covariates, and exclusion of outliers 247 exposed workers and 139 controls (NCI study) and 114 exposed workers and 50 controls (NYU study) remained. In the NYU study, information on urinary metabolites CA and HQ was not available for 97 exposed workers and 25 controls. Descriptive statistics for both study populations are presented in Table 1.

A uniform background benzene exposure level for control workers was predicted based on the average urinary benzene concentrations among the controls of the NCI study, using a calibration curve obtained from benzene exposed subjects as described by Kim et al. (2006). This resulted in a background benzene air concentration level of 0.003 ppm.

Generalized additive models (GAMs) with the use of smoothing splines were fitted to the pooled data using the MGCV package in R. GAMs extend the linear parametric model by allowing linear functions of the predictor variable to be replaced by a smooth function permitting a more flexible evaluation of the ERC without applying a priori assumptions regarding the shape of the ERC. To account for different effects between the studies, an interaction term between the smoothing parameter (penalty) and study was included. Smoothing parameters were selected by generalized cross validation (GCV). Lower bounds for the smoothing parameters were supplied when necessary to achieve monotonicity. First, we analyzed log-transformed WBC counts and its major sub-populations (granulocytes, monocytes, and lymphocytes) against personal air benzene exposure estimates (untransformed) (i.e. log-linear exposure response model). Log transformation of the endpoints was done to deal with the (potential) non-homogeneous residual variation. Secondly, to obtain insight if non-linear features of the exposure response associations could be explained by metabolic saturation we repeated the above analyses for WBC counts and the urinary metabolites PH, HQ, CA and MA. These metabolites had previously shown effects of saturable benzene metabolism resulting in proportional less metabolites being formed at higher exposure levels (Kim et al., 2006).

To obtain a more comprehensive analysis of the relationship between air benzene and WBC counts we performed quantile regression in which the effect of benzene exposure on the 10th, 25th, 50th, 75th, 90th percentile of log-transformed WBC counts was modeled. Whereas ordinary least-squares regression models describe the relation between a covariate X and the conditional mean of the response variable Y, in quantile regression models the relation between X and the conditional quantiles of Y are modelled. Akin to the GAM analyses we allowed for non-linearity in these associations by fitting a natural-spline function as available in the QUANTREG procedure in R.

We have previously shown that having a total WBC blood count < 4000/μl measured repeatedly over several months is associated with a strong increased risk of subsequently developing a hematological malignancy (Rothman et al., 1997). We therefore modeled the exposure response relationship between benzene exposure and risk of having a WBC count < 4,000/μl. Risk [odds ratio with 95% confidence intervals (CI)] was modeled using a GAM logistic regression model.

All analyses were adjusted for age, sex, current cigarette smoking and study if applicable. To examine the influence of missing observations in CA and HQ, we performed sensitivity analyses of all main analyses, excluding all participants with missing observations for these metabolites. All statistical analyses were conducted with R, version 3.2.5 (R Core Development Group, Vienna, Austria).

3. Results

Descriptive characteristics of the two study populations are given in Table 1. On average the NYU study population was slightly older as compared to the NCI study. Benzene exposure was higher in the NYU than the NCI study (average 10.0 and 4.9 ppm, median 3.7 and 1.2 ppm, respectively). However, the exposure range largely overlapped (min–max 0.08–212 and 0.19–78.2 ppm for the NYU and NCI study respectively). These differences were reflected in the levels of benzene metabolites CA, HQ, and PH, which were significantly higher in the NYU exposed population as compared to the exposed population in the NCI study. In contrast MA was lower in the NYU-study as compared to the NCI-study. Metabolite levels of HQ and PH were also significantly higher in the unexposed NYU population than in the unexposed NCI population. No differences in peripheral blood cell counts were observed between the unexposed workers in the two studies.

Spearman correlations between air benzene concentrations and urinary metabolites ranged between 0.50 for CA and 0.82 for MA among the exposed subjects of the NCI study. For the NYU exposed population, the correlations between air benzene concentrations ranged between 0.29 for CA and 0.76 for MA. In the unexposed population, correlations between urinary metabolites were somewhat stronger within the NYU study (range: 0.42–0.78) than the NCI study (range: 0.19–0.44) (Table S1).

Table 1
Descriptive characteristics of the two study populations.

NCI-Study				**NYU-Study**					
		Unexposed (n = 139)		Exposed (n = 247)		Unexposed (n = 50)		Exposed (n = 114)	
Demographics									
Age	Yrs (Mean, SD)	30.3	8.7	29.5	8.3	33.3	7.5	36.0	7.8
Gender	Male (N, %)	52	37.4	85	34.4	24	48.0	59	51.8
	Female (N, %)	87	62.6	162	65.6	26	52.0	55	48.2
Current smokers	Yes (N, %)	39	28.1	51	20.6	10	20.0	37	32.5
	No (N, %)	100	71.9	196	79.4	40	80.0	77	67.5
Benzene exposure									
Air benzene	ppm (Mean, SD)	0.003[A]	N.A.	4.88	11.3	0.003 [A]	N.A	9.96	22.2
Phenol	μM (Mean, SD)	74.7	54.7	284.4	424.9	92.7	209.9	358.6	665.0
Hydroquinone [B]	μM (Mean, SD)	7.9	5.4	36.8	57.6	43.5	73.2	301.8	187.0
Muconic Acid	μM (Mean, SD)	1.4	1.2	33.8	61.3	1.7	1.9	31.2	41.1
Catechol [B]	μM (Mean, SD)	14.5	9.6	32.0	42.5	27.0	28.8	194.3	168.9
Peripheral blood cell counts									
White blood cells	Cells/μl (Mean, SD)	6473	1713	5515	1386	6682	1515	6111	1442
Granulocytes	Cells/μl (Mean, SD)	4100	1408	3352	1078	4042	1269	3386	1123
Lymphocytes	Cells/μl (Mean, SD)	2129	579	1946	535	2391	837	2500	745
Monocytes	Cells/μl (Mean, SD)	241	92	217	95	248	142	226	141

[A] A uniform background benzene exposure level for control workers was predicted based on the average urinary benzene concentrations among the controls.
[B] In the NYU study, information on urinary metabolites catechol and hydroquinone was not available for 97 exposed workers and 25 controls.

Fig. 1 shows the overall exposure response relationship between air benzene concentrations and WBC counts (Panel A) and by study (Panel B). Overall, and in each of the study separately, we observe a steep decrease in WBC counts at the lower range of benzene exposure (see inserts magnifying the range between 1 and 10 ppm) with a departure from linearity at relatively low benzene levels (<10 ppm) and a flattening of the ERC at air benzene concentrations > 50 ppm. Sensitivity analyses showed no clear differences in results when untransformed WBC counts were used in the analyses showing that the observed non-linear association is not driven by the log-transformation of cell counts (results not shown).

A similar exposure–response pattern was observed for the major constituents of WBC granulocytes, and lymphocytes but not for monocytes (Fig. 2). The later was mainly caused by a difference in response in the NYU study for monocytes (see online material for results by study; Figure S1). The non-linearity in the ERCs seemed to be most pronounced for WBC, followed by granulocytes and lymphocytes, as judged by the degrees of freedom (d.f.) required for the smoothing parameter were 2.80, 2.51, and 1.4, respectively; where d.f. = 1 indicates a linear association.

We subsequently explored the association between WBC counts and the benzene urinary metabolites that have previously shown saturation effects (Fig. 3) (Kim et al., 2006). All analyses showed a relatively similar effect as was seen for air benzene with an initial drop in WBC counts and a subsequent flattening of the ERC. The non-linearity in the ERCs seemed to be more pronounced for CA, HQ, PH as compared to MA, based on the degrees of freedom (d.f.) of the smoothing parameter being respectively 2.84, 2.70, 2.45 and 1.58, respectively. Analyses stratified by study showed largely similar effects except for PH which showed a linear effect in the NYU study while a non-linear effect was observed in the NCI study (see online material; Figure S2). To facilitate direct comparisons of the results of air benzene and its metabolites we plotted the results in a single graph by standardizing the effect of benzene and its metabolites on WBC counts by a change in standard deviation in air benzene exposure or its metabolites (Fig. 4). These results indicate similar shapes for air benzene and metabolites on WBC apart from MA which seems to plateau at a slightly higher WBC count.

Quantile regression using a natural spline showed essential parallel exposure response curves for the 10th, 25th, 50th, 75th, 90th percentile of log-transformed WBC counts (Fig. 5). However, on an absolute scale a larger drop in cell counts was observed in the 90th percentile compared to the 10th percentile resulting in a decrease in variance at higher air benzene exposures. Results of the 50th percentile were consistent with the result of the Generative Additive Model as displayed in Fig. 1.

ERC analysis between air benzene exposure levels and risk of having a WBC count less than 4,000cells/μl showed a monotonic exposure–response relation with no indication of a threshold or flattening of the exposure–response curve at higher levels of exposure albeit that confidence intervals are wide (Fig. 6). When benzene exposure was modeled in categories of < 1 ppm, 1–10 ppm and > 10 ppm for exposed participants, increased risks for < 1 ppm (OR: 2.93, 95%CI: 1.17–8.03), 1–10 ppm (OR: 5.50, 95%CI: 1.82–17.09) and > 10 ppm (OR: 3.73, 95%CI: 1.57–9.88) were observed when compared with workers exposed to ambient (ppb) levels of benzene. Similar ERCs were observed when the risk was modeled for benzene metabolites (see online material; Figure S3).

Sensitivity analyses showed no clear differences in results when participants with missing data for CA or HQ were excluded from all other analyses (results not shown).

4. Discussion

There is continuing debate on the shape of the exposure–response association of benzene's carcinogenic and biological effects. We and others have previously postulated that the association between benzene and hematological effects may not be linear (Lan et al., 2004; Vlaanderen et al., 2010; McHale et al., 2008; Rappaport et al., 2010; Vlaanderen et al., 2011). In a *meta*-regression on benzene-leukemia risk, we showed evidence for a possible proportional higher risk at relatively low cumulative levels of benzene exposure than at higher cumulative benzene exposure levels (Vlaanderen et al., 2010). An observation that is supported by evidence on saturation of metabolic pathways at higher levels of benzene exposure resulting in a decrease in the rate of (toxic) metabolite and adduct formation (Kim et al., 2006; Lin et al., 2007). We also previously described non-linear responses in benzene induced hematotoxicity with a larger than proportional decrease in cell counts at

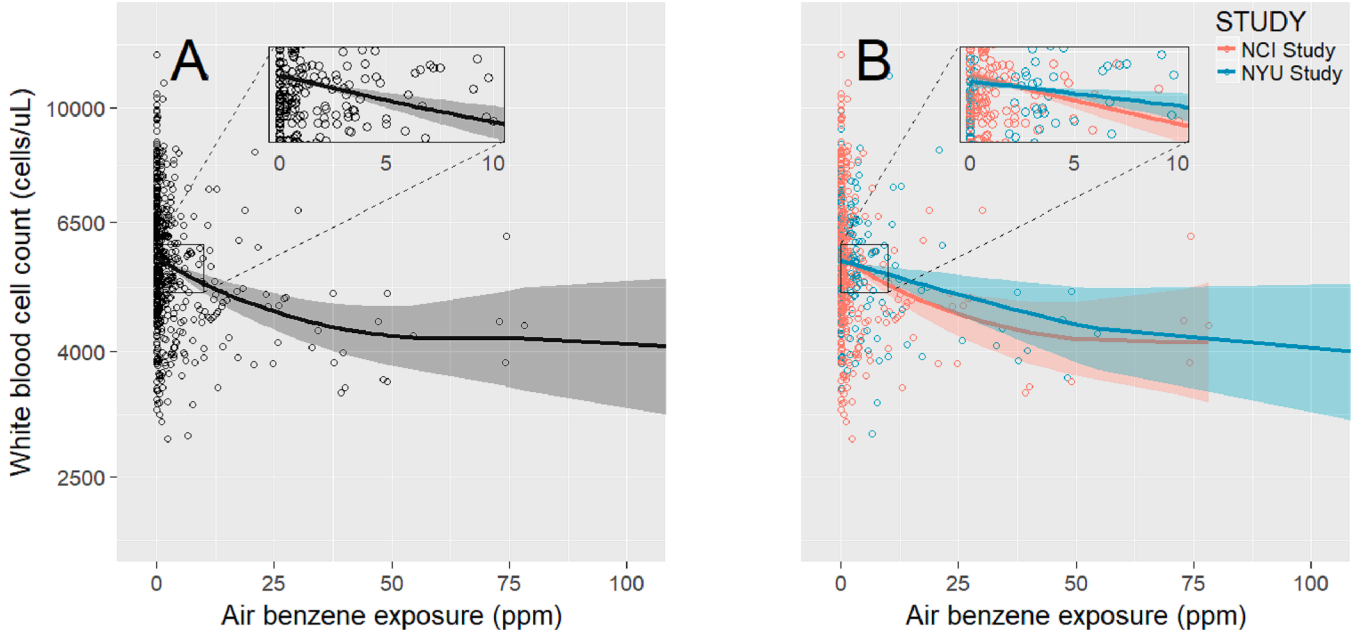

Fig. 1. White blood cell counts (cells/μl) in relation to air benzene exposure level (ppm) of the pooled study population (panel A) and by study (panel B). Solid line is the smoothed log-linear exposure–response function. Shaded areas are the corresponding 95%-confidence intervals. For presentation purposes the X-axis was truncated at 100 ppm benzene (~97.5 percentile of the exposed distributions). Inserts depict the exposure range up to 10 ppm.

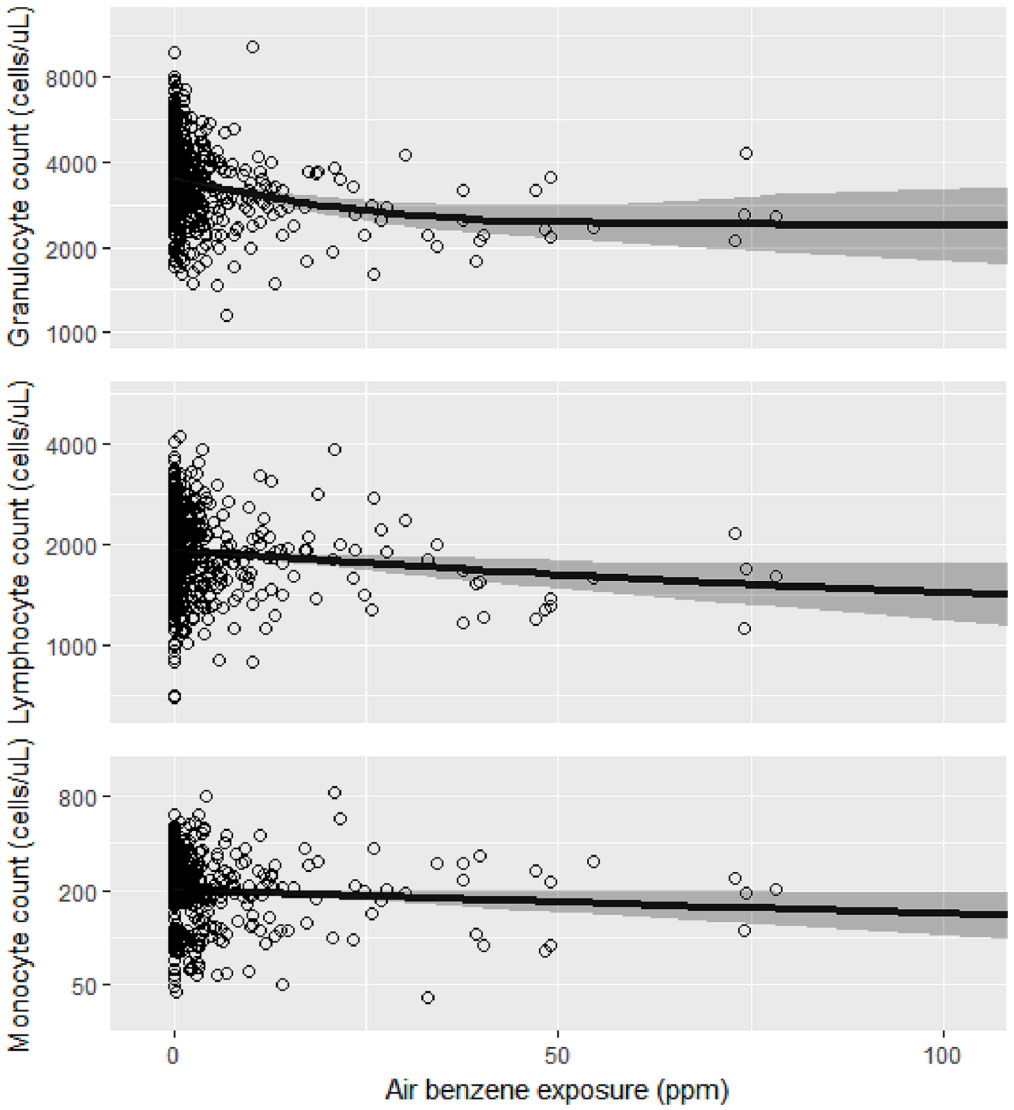

Fig. 2. Major white blood cell sub-populations (cells/μl) in relation to air benzene exposure (ppm). Solid line is the smoothed log-linear exposure–response function. Shaded areas are the corresponding 95%-confidence intervals. For presentation purposes the X-axis was truncated at 100 ppm benzene (~97.5 percentile of the exposed distributions).

lower than at higher levels of benzene (Lan et al., 2004).

Here we describe a pooled analyses of two independent cross-sectional studies that used comparable methods, conducted in Tianjin China in 2000/2001. We show that the pooled and study-specific ERCs are non-linear with air benzene exposure with a less than proportional decrease in WBC counts at higher levels of benzene exposure. Our derived benzene-WBC ERC fits the observed effect on WBC at the higher end of air benzene exposure levels from a separate study published in 1996 among Chinese exposed workers where we would predict, at a benzene concentration of 100 ppm, a decrease in WBC counts of 1,600 cells/μl while a drop of 1,400 cells/μl was observed (Rothman et al., 1996).

Our derived ERC between benzene and hematological effects suggests no strong evidence of a threshold among the exposed workers. Both cross-sectional studies previously reported evidence of hematotoxicity at levels below 1 ppm. Qu et al. (2002), (NYU study) reported that at < 0.5 ppm air benzene concentrations, decreases in WBC counts were observed while in the study of (Lan et al., 2004) (NCI study), significant decreases in WBC counts were observed at < 1 ppm air benzene concentrations. The latter results extended to a subset of workers exposed to ~ 0.3 ppm benzene and with negligible exposure to other solvents. Together these observations provide no evidence of a threshold in benzene induced hematotoxicity down to at least $\pm$ 0.1/0.2 ppm which were the minimum level that could be detected by the air monitors in our studies.

We further explored if saturation of the metabolic pathways could explain the observed non-linear association between air benzene exposures and decreases in the WBC and its major sub-populations. These analyses showed that the non-linear exposure–response associations persisted if urinary concentrations of PH, CAT, MA and HQ were used instead of air benzene concentrations. It therefore seems that saturation in metabolism as represented by the measured urinary metabolites does not fully explain the flattening observed in the ERC especially at higher levels of benzene exposure (>50 ppm). This parallels observations from hematological studies of mice after exposure to inhaled benzene that also showed evidence for supra-linear associations between WBC and benzene exposure and although the magnitude of the effects was modified by metabolic factors (i.e. NAD(P)H: quinone oxidoreductase-1 (NQO1)) the shape of the exposure–response curve was not (Bauer et al., 2003). This observation however does not preclude that metabolic saturation plays a role in the departure from linearity observed at lower levels of benzene which has been shown to occur in this exposure range

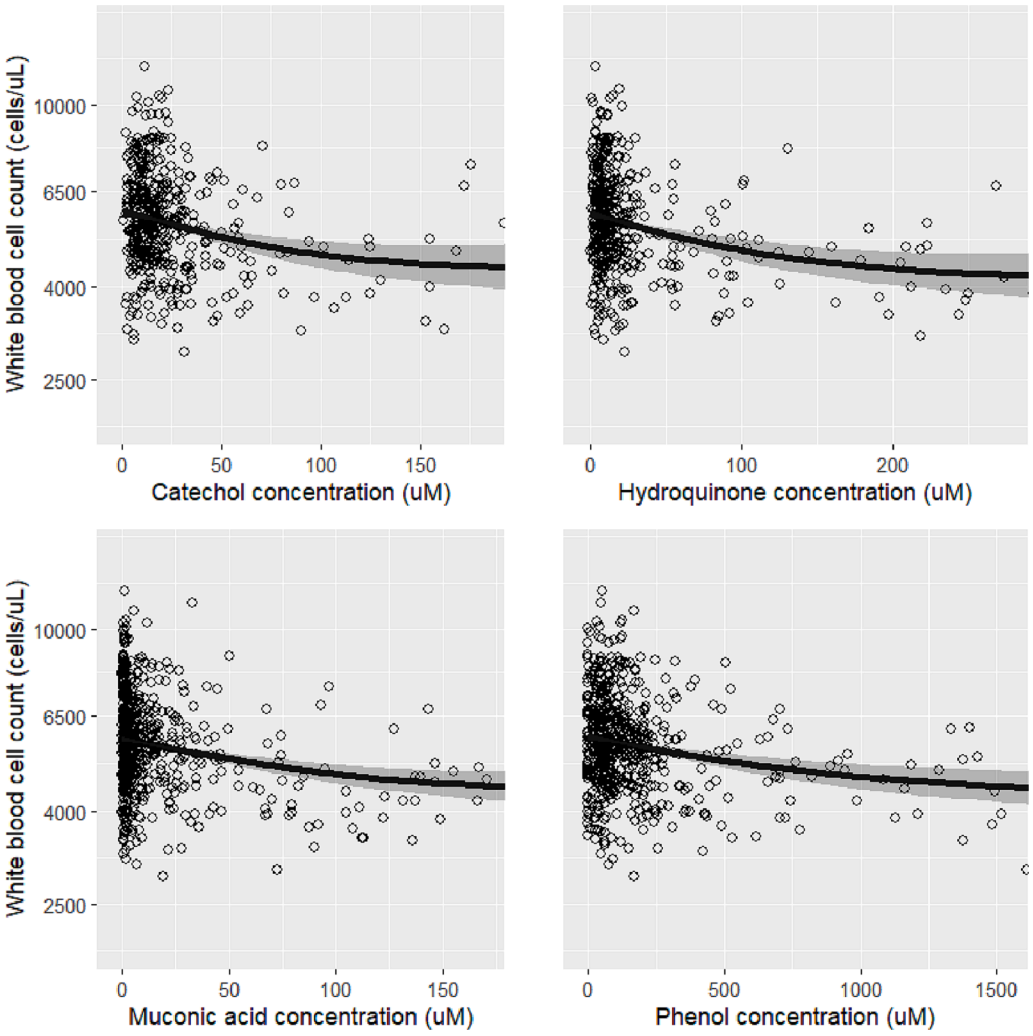

Fig. 3. White blood cell counts (cells/μl) in relation to major benzene metabolite levels (μM). Solid line is the smoothed log-linear exposure–response function. Shaded areas are the corresponding 95%-confidence intervals. For presentation purposes the X-axis was truncated at the 100 ppm air benzene equivalent (~97.5 percentile of the exposed distributions).

(Kim et al. (2006)).

Our analyses indicate that the ERC with air benzene or its metabolites departs from linearity at benzene air concentrations < 10 ppm and bottoms at around 4000 cell/μl at benzene concentrations > 50 ppm. Additional, quantile analyses showed that the decrease, in absolute cell counts, was more pronounced for the 90th and 75th percentile than they were for the 25th and 10th percentile leading to less variance in WBC counts at higher benzene exposure levels. These results resemble observations of the radiation and antineoplastic literature where after exposure a sharp drop in WBC is observed, followed by a nadir, and subsequently a restoration period (Raghavendran et al., 2012; Vorobiev, 1997). This restoration period is characterized by an increase in the proliferation rate of HSCs. We observed however that some individuals had WBC counts less than 4000cells/μl which already occurred at relatively low benzene levels as well as high benzene levels. Studies by Aksoy among heavily exposed workers to benzene (between 30 and 210 ppm) have shown that less than 10% of exposed individuals had WBC counts lower than 4000 cells/μl and that thus a majority of heavily exposed subjects maintained counts above 4000 cells/μl (Aksoy et al., 1971; Aksoy et al., 1987). This is consistent with our observation that even at high benzene exposure levels the majority of subjects will not have blood cell counts below 4000cells/μl.

As having a total WBC count < 4000/μl measured repeatedly over several months has been associated with a strong increased risk of developing a hematological malignancy, we additionally investigated the risk of having a blood cell count lower than 4000cells/μl within our pooled cross-sectional data. Noting that having a (potential) transient count below 4000cells/μl cannot be equated directly to the evidence on benzene poisoning and hematological malignancy. These analyses, in contrast to the modeling of continuous WBC counts, showed a linear ERC with air benzene exposure and major urinary metabolites without strong evidence of a plateauing effect albeit that power is limited. Based on basic principles of bone marrow biology, a decrease of the WBC count to 4000cells/μl or less in the peripheral blood can be reflective of the bone marrow failing to provide white cells to the peripheral through normal differentiation and maturation (Raghavendran et al., 2012; Vorobiev, 1997). Indeed, such alterations are commonly seen in patients exposed to medications with well-documented bone marrow toxic properties. In reviewing our results and prior published studies, we propose that a WBC count of less than 4000cells/μl in individuals exposed to benzene is reflective of disrupted differentiation and maturation of the bone marrow. This might explain some biological observations that have been described previously among benzene exposed workers. Studies among benzene poisoning patients have described both hypo- and hyper cellularity in bone marrow of patients chronically exposed to benzene (Aksoy et al., 1972; Ruiz et al., 1994). This may be indicative of an initial response of increased cell proliferation leading to hyperplasia of the bone marrow, which turns into hypoplasia when the

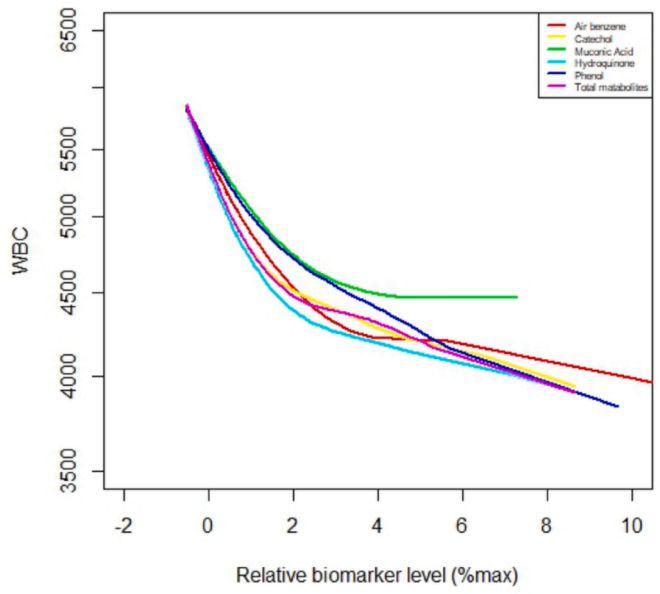

Fig. 4. White blood cell counts (cells/μl) in relation to air benzene and major benzene metabolite levels expressed as a change in WBC counts per change in standard deviation of the exposure distribution.

restorative mechanism fails. Similar as to the dynamics of radiation induced hematotoxicity, this could be due to death of damaged HSCs in combination of depletion of the undamaged HSC reservoir and toxicity to the stromal environment (Smirnova et al., 2014). Alterations that increase the replicative potential of HSCs could lead to clonal expansion during hematopoietic recovery (Stanley et al., 2017).

We do note that there are several studies on low benzene exposure in the chemical industry in Western countries, which overall find no evidence of hematotoxicity (Collins et al., 1991; Collins et al., 1997; Swaen et al., 2010; Tsai et al., 2004). However, these studies had one or more limitations including that they were not purposely designed to study subtle hematological effects and used routinely collected hematological and exposure data. The exposure assessment in these studies was based on routine sampling strategies targeted at jobs, workplaces and times where exposure was expected. The job and task-based approach, instead of purposely designed monitoring strategies using individual full-shift measurement data, may have resulted in exposure misclassification which in combination with the low benzene exposure levels observed in these industries may have masked a possible effect. An alternative explanation would be that Caucasians are less susceptible to hematological effects of benzene than Asians but no direct evidence for this has been provided to date. Our previous *meta*-regression on benzene induced leukemia also provides no strong evidence for this association to be different between these populations (Vlaanderen et al., 2010).

We used controls from the same geographical region which were frequency matched by sex and age. We observed no differences in WBC counts between controls of the two studies that were recruited from in total 5 different factories and different departments. Although low occupational dust exposures might have occurred (e.g. textile dust) in some departments of selected control factories the comparability of the WBC counts between the controls of the two studies indicate that such exposures did not lead to increases in WBC counts. We estimated the background exposure levels of the controls based on the average urinary benzene concentration at 0.003 ppm. As the ERC was modeled in natural space the exact assignment of background levels has limited influence on the derived exposure response association. Analyses without controls resulted in similar ERCs (see online material; Figure S4).

In conclusion, we present evidence for a non-linear exposure response association between air benzene exposure and hematological effects without any obvious indication of a functional threshold at the exposure levels studied (0.1 to 100 ppm). Furthermore, we found evidence that the flattening of the ERC at higher benzene exposure levels (>50 ppm) is not likely caused by metabolic saturation of benzenes metabolism, as represented by the major urinary metabolites. We pose that rather than due to saturable metabolism, the observed flattening of the ERC especially at higher benzene exposure levels could be due to a response by the bone marrow to maintain hematopoietic homeostasis. Toxicity to the bone marrow and an induced hyperproliferative response could both contribute to risk of subsequently developing a hematopoietic malignancy. Additional work is needed to explore this hypothesis.

CRediT authorship contribution statement

Roel Vermeulen: Conceptualization, Methodology, Investigation,

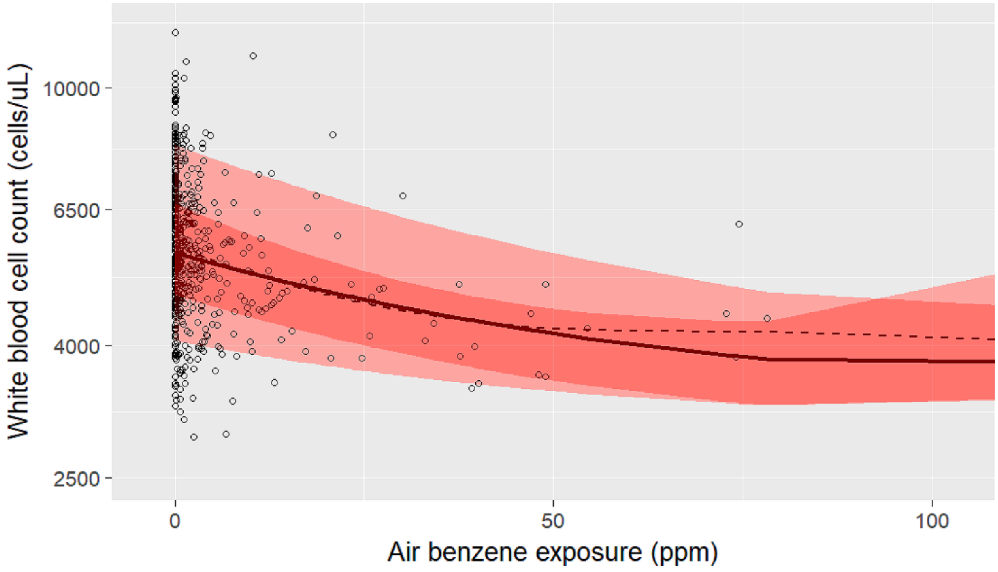

Fig. 5. Quantile regression (5th, 10th, 50th, 90th, 95th) of white blood cell counts (cells/μl) in relation to air benzene exposure (ppm) using a natural-spline (dashed line). For comparison, the result of the General Additive Model (solid line) and 95% confidence interval is also displayed. For presentation purposes the X-axis was truncated at 100 ppm air benzene (~97.5 percentile of the exposed distributions).

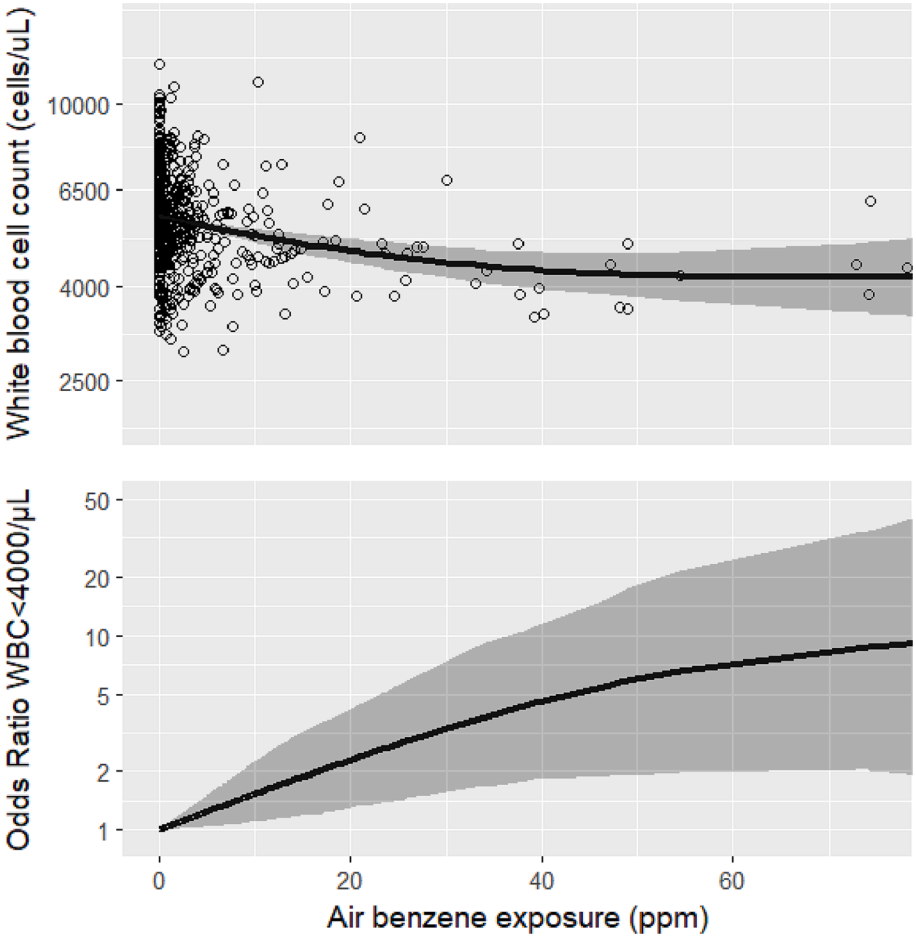

Fig. 6. White blood cell counts (cells/µl) in relation to benzene exposure level (ppm) of the pooled study population (similar as to Fig. 1 panel A) together with the odds ratio (OR) of having a white blood cell count < 4000 cells /µl in relation to benzene exposure (ppm). The solid lines are smoothed log-linear exposure–response functions. Shaded areas are the corresponding 95%-confidence intervals (CI). For presentation purposes the X-axis was truncated at 75 ppm benzene, which is the highest exposure at which a case occurred.

Formal analysis, Writing – original draft. **Qing Lan:** Conceptualization, Writing – review & editing, Resources. **Qingshan Qu:** Conceptualization, Writing – review & editing. **Martha S. Linet:** Writing – review & editing. **Luoping Zhang:** Investigation, Writing – review & editing. **Guilan Li:** Investigation, Writing – review & editing. **Lutzen Portengen:** Methodology, Formal analysis, Writing – original draft. **Jelle Vlaanderen:** Writing – review & editing. **Kim Sungkyoon:** Writing – review & editing. **Richard B. Hayes:** Writing – review & editing, Conceptualization. **Songnian Yin:** . **Martyn T. Smith:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Stephen M. Rappaport:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Nathaniel Rothman:** Conceptualization, Methodology, Investigation, Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108007.

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