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## ORIGINAL MANUSCRIPT

# Comparison of hematological alterations and markers of B-cell activation in workers exposed to benzene, formaldehyde and trichloroethylene

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## Abstract

Benzene, formaldehyde (FA) and trichloroethylene (TCE) are ubiquitous chemicals in workplaces and the general environment. Benzene is an established myeloid leukemogen and probable lymphomagen. FA is classified as a myeloid leukemogen but has not been associated with non-Hodgkin lymphoma (NHL), whereas TCE has been associated with NHL but not myeloid leukemia. Epidemiologic associations between FA and myeloid leukemia, and between benzene, TCE and NHL are, however, still debated. Previously, we showed that these chemicals are associated with hematotoxicity in cross-sectional studies of factory workers in China, which included extensive personal monitoring and biological sample collection. Here, we compare and contrast patterns of hematotoxicity, monosomy 7 in myeloid progenitor cells (MPCs), and B-cell activation biomarkers across these studies to further evaluate possible mechanisms of action and consistency of effects with observed hematologic cancer risks. Workers exposed to benzene or FA, but not TCE, showed declines in cell types derived from MPCs, including granulocytes and platelets. Alterations in lymphoid cell types, including B cells and CD4+ T cells, and B-cell activation markers were apparent in workers exposed to benzene or TCE. Given that alterations in myeloid and lymphoid cell types are associated with hematological malignancies, our data provide biologic insight into

the epidemiological evidence linking benzene and FA exposure with myeloid leukemia risk, and TCE and benzene exposure with NHL risk.

### Abbreviations

BMI	body mass index
FA	formaldehyde
MPC	myeloid progenitor cell
NHL	non-Hodgkin lymphoma
TCE	trichloroethylene
WBC	white blood cell

## Introduction

Benzene, formaldehyde (FA) and trichloroethylene (TCE) are ubiquitous chemicals used occupationally for various production and manufacturing purposes and are commonly present in the general environment. Benzene is used as a chemical intermediate, as a solvent for organic materials, and during the manufacturing of rubbers, dyes and other products, whereas FA is used as a preservative, in the production and use of resins, and as an intermediate in the production of other industrial chemicals. Benzene and FA are found in cigarette smoke and automobile emissions, and FA is present in a variety of common consumer and household products (1,2). TCE is used in various industries as a vapor degreaser and general purpose solvent, and has been detected in about one-third of municipal water supplies in the USA (3).

These three chemicals have been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (4,5), and have been evaluated by other agencies including the U.S. Environmental Protection Agency and the National Academy of Sciences (6–9). Much focus has been placed on elucidating the association between these chemicals and lymphohematopoietic malignancies (LHMs), including for myeloid leukemia and non-Hodgkin lymphoma (NHL) (10–12). Benzene is an established risk factor for acute myeloid leukemia (AML) and there is some evidence for a role of benzene in the development of NHL, based on results from some case–control studies and occupational cohorts (13). However, the association with NHL is not conclusive, potentially due to the etiologic heterogeneity of NHL subtypes and changes in disease classification over time (12). TCE is a Group 1 carcinogen based on sufficient evidence for an association with renal cell carcinoma and has also been associated with NHL, although the epidemiologic evidence is inconclusive (5). A case–control study with detailed exposure assessment conducted in the USA reported that TCE at high exposure levels may increase NHL risk, a finding that is consistent with some occupational cohorts, but differences across studies with respect to exposure assessment approaches and inability to evaluate NHL subtype-specific associations may contribute to inconsistent findings in the literature (14,15). A recent meta-analysis of occupational TCE exposure and risk of lymphohematopoietic cancers supported an association between TCE and NHL risk (16).

The most recent IARC assessment of FA concluded that there is now sufficient evidence for an association with leukemia, particularly the myeloid subtype, based on epidemiologic evidence and additional mechanistic evidence (4). Taken together, the current epidemiologic evidence suggests that benzene is an

established myeloid leukemogen and probable lymphomagen, whereas FA was recently classified as a human myeloid leukemogen but has not been associated with NHL (4). Although the association has been the subject of debate, TCE is a probable lymphomagen based on observed associations with NHL, but has not been observed to be associated with risk of myeloid leukemia (5).

We have previously conducted three cross-sectional molecular epidemiology studies in China evaluating the early biologic effects of benzene, TCE and FA among healthy factory workers in Tianjin and Guangdong (17–19). We have reported in separate, chemical-specific individual papers the data presented here on peripheral blood cell counts from a complete blood count and the major lymphocyte subsets for benzene (18), TCE (17) and FA (19), on subsets of CD4<sup>+</sup> and CD8<sup>+</sup> T cells for TCE (20) and FA (21), on aneuploidy of chromosome 7 in myeloid progenitor cells (MPCs) for benzene (22) and FA (23) and on concentrations of soluble CD27 (sCD27) and CD30 (sCD30) for TCE exposure (17). Given the current epidemiologic evidence suggesting that each of these chemicals are associated with specific LHMs but not others, here we compare and contrast the patterns of hematotoxicity for each of these chemicals. We further present previously unpublished data on sCD27 and sCD30, two markers indicative of B-cell activation, in relation to benzene and FA exposure in order to provide insight into the biologic plausibility for the disease specific findings. CD27 and CD30 are members of the tumor necrosis factor superfamily that are expressed on subsets of lymphocytes, including B-cells, and have broad immunoregulatory functions including regulation of lymphocyte activation. Higher levels of sCD27 and sCD30 have been strongly associated with future risk of NHL in recent prospective studies (24), including among individuals from China (25). These associations have notably been observed over 10 years before NHL diagnosis, suggesting that the markers themselves and/or the underlying process that they represent may contribute to lymphomagenesis.

## Materials and methods

### Study designs and exposure assessments

Detailed information regarding the study design, exposure assessment protocols, and hematological results for each of the three cross-sectional studies has been described (17–21). Briefly, the benzene study was conducted in Tianjin, China, and included 250 exposed workers from two shoe manufacturing facilities and 140 unexposed workers from other factories. Personal benzene exposure was repeatedly monitored using a 3M organic vapor passive monitoring badge worn by workers for a full work shift for a period up to 16 months before phlebotomy.

The TCE exposure study was conducted in Guangdong, China, and included 80 exposed workers in six factories and 96 unexposed workers matched by sex and age ( $\pm 5$  years) from separate factories in the same geographic region that did not use TCE in the manufacturing process, including two clothes manufacturing factories, one food production factory and a hospital. Two to three personal air exposure measurements using a 3M organic vapor monitor 3500 were collected for all exposed workers. These measurements were collected from exposed workers over the course of a full work shift during a 3-week period before blood collection, and the arithmetic mean of the exposure measurements in each worker was used to categorize subjects into lower and higher exposed groups based on the overall median TCE exposure level (i.e. 12 p.p.m.).

The FA exposure study was conducted in Guangdong, China, and included 43 exposed workers in two factories that used or manufactured melamine and a subset of unexposed workers from the TCE study ( $n = 51$ ) that were matched to the exposed workers by sex and age ( $\pm 5$  years). Personal FA exposure was monitored with SKC UME<sub>x</sub> 100 passive samplers, which were worn by workers in the exposed factories for a full work shift for about three workdays over a 3-week period.

Subjects from all three studies were administered a questionnaire that sought information on occupational and medical history, environmental exposures, and current tobacco and alcohol use. In addition, blood samples and other biologic specimens were collected from all workers. Informed consent was obtained from all subjects, and the studies were approved by Institutional Review Boards at the U.S. National Cancer Institute, the Guangdong National Poison Control Center and the Chinese Center for Disease Control and Prevention.

### Biological samples and laboratory analysis

Blood and post-shift urine samples from subjects in the benzene study were collected and delivered to the processing laboratory within 6 h. The complete blood count and differential were analyzed using a Beckman-Coulter® T540 blood counter, and lymphocyte subsets were measured using a FACS Calibur flow cytometer (Software: SimulSET v. 3.1). Samples were obtained from subjects in the FA and TCE studies at the time the questionnaire was administered, and included peripheral blood and buccal cell samples in addition to post-shift and overnight urine samples. Blood samples were sent to processing laboratories within 6 h and were analyzed using a Sysmex XT-1800i automated hematology analyzer. Subsets of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells for the TCE and FA studies were measured using a flow cytometric procedure that has been described in detail (20,21). To evaluate the effect of benzene and FA exposure on aneuploidy of chromosome 7 in MPCs, colonies that give rise to granulocytes and macrophages (CFU-GM) were cultured from mononuclear cells as described previously (23). Aneuploidy of chromosome 7, a cytogenetic abnormality frequently associated with myeloid leukemia, was subsequently examined in a subset of the subjects from the benzene (28 exposed, 14 control) and FA (29 exposed, 23 control) studies using FISH (22,23). For all three studies, plasma sCD27 and sCD30 were measured using enzyme-linked immunosorbent assay (Bender Medsystems, Vienna, Austria). Exposed and control workers were assayed consecutively in the same batch.

### Statistical analysis

Previously conducted analyses have been described (17–22). Briefly, unadjusted means and standard deviations were calculated for all endpoints. Linear regression using the natural logarithm of each endpoint was used to test for differences between workers exposed to benzene, FA and TCE,

and unexposed workers. Benzene air levels used in the analyses were based on the arithmetic mean of an average of two measurements per subject collected during the month before phlebotomy. Negative binomial regression was used to analyze monosomy 7 data. All of the regression models were adjusted for age (continuous variable) and sex. Further adjustment for current cigarette smoking (yes/no), current alcohol consumption (yes/no), recent respiratory infection (yes/no) and body mass index (BMI) was conducted if the regression coefficient of the corresponding exposure variable (i.e. benzene, TCE and FA) was altered by  $\pm 15\%$ . A linear trend test for cell counts was conducted using an ordinal variable for TCE exposure and natural log-transformed air levels for benzene exposure to assess a potential exposure–response effect across exposure categories. Analyses for FA were conducted for exposed versus unexposed only as the exposed group was selected on the basis of having high exposures, and as a consequence the exposure levels in this study were relatively homogenous. For the soluble CD27 and CD30 analyses, which have not previously been published for benzene and FA, exposure categories included exposed versus unexposed for FA and  $<10$  and  $\geq 10$  p.p.m. in relation to unexposed workers for benzene, with linear regression models adjusted for age and sex as well as additional variables (i.e. smoking, alcohol consumption, recent infection and BMI) using the same criteria as described above for other endpoints. All statistical analyses were conducted using Statistical Analysis Software (SAS v. 9.1.3, Cary, NC).

## Results

### Demographic characteristics and exposure levels

Demographic and lifestyle characteristics of subjects from the three studies are shown in Table 1. Subjects in the FA and TCE studies were predominately male (86% and 74%, respectively), whereas the majority of the workers in the benzene study were female (65%). The mean ages of workers in the three studies ranged from 25 to 31 for exposed workers and 27 to 30 for control workers. The majority of workers in each study did not smoke cigarettes or consume alcohol, and most did not report having a recent respiratory infection (Table 1). The median (10th, 90th percentiles) air exposure levels of benzene, FA and TCE in exposed workers were 1.2 p.p.m. (0.3, 12.4), 1.3 p.p.m. (0.6, 2.5) and 12.1 p.p.m. (1.8, 43.1), respectively (Table 1).

### White blood cell counts

Overall white blood cell (WBC) counts were significantly decreased in workers exposed to each of the three chemicals

**Table 1.** Selected characteristics of unexposed workers and workers exposed to benzene, FA and TCE in three cross-sectional molecular epidemiology studies conducted in China

Characteristic	Benzene		Formaldehyde		TCE	
	Control	Exposed	Control	Exposed	Control	Exposed
	$n = 140$	$n = 250$	$n = 51$	$n = 43$	$n = 96$	$n = 80$
Sex (n, %)						
Male	52 (37)	86 (34)	44 (86)	37 (86)	73 (76)	57 (71)
Female	88 (63)	164 (66)	7 (14)	6 (14)	23 (24)	23 (29)
Mean Age (SD)	30 ( $\pm 9$ )	30 ( $\pm 8$ )	30 ( $\pm 7$ )	31 ( $\pm 6$ )	27 ( $\pm 7$ )	25 ( $\pm 7$ )
Mean BMI (SD)	23 ( $\pm 4$ )	22 ( $\pm 3$ )	22 ( $\pm 3$ )	21 ( $\pm 3$ )	22 ( $\pm 3$ )	21 ( $\pm 3$ )
Current smoking (n, %)						
Yes	39 (28)	52 (21)	23 (45)	18 (42)	38 (40)	34 (42)
No	101 (72)	198 (79)	28 (55)	25 (58)	58 (60)	46 (58)
Current alcohol (n, %)						
Yes	43 (31)	67 (27)	21 (41)	11 (26)	40 (42)	26 (32)
No	97 (69)	183 (73)	30 (59)	32 (74)	56 (58)	54 (68)
Recent infection (n, %)						
Yes	16 (11)	18 (24)	15 (29)	17 (40)	21 (22)	15 (19)
No	124 (89)	232 (76)	36 (71)	26 (60)	75 (78)	65 (81)
Median exposure, p.p.m. (10th, 90th percentile)	—	1.2 (0.3, 12.4)	—	1.3 (0.6, 2.5)	—	12.1 (1.8, 43.1)

at varying exposure levels, compared with unexposed workers (Figure 1A). Whereas benzene exposure was associated with an exposure-dependent decline in WBC counts ( $P_{\text{trend}} < 0.001$ ), including about a 15% decline for exposures  $<1$  p.p.m. and a 26% decline in workers exposed to  $\geq 10$  p.p.m. compared with unexposed workers (Figure 1A), a decline in WBCs was apparent in TCE-exposed workers only for exposures  $\geq 12$  p.p.m. ( $P = 0.03$ ), and no exposure-response relationship was observed (Figure 1A). Workers exposed to FA had a significant decline in WBC counts compared with unexposed factory workers (13%;  $P < 0.01$ ) (Figure 1A).

### Cells of myeloid lineage

The effects of each chemical on cell types derived from the myeloid lineage are shown in Figure 1A. Whereas workers exposed to TCE showed no evidence of a decline in either granulocytes,

platelets or monocytes, workers exposed to benzene and FA had significant declines in granulocytes and platelets and exposure-dependent decreases of these cell types were associated with benzene exposure ( $P_{\text{trend}} < 0.001$ ; Figure 1A). Further, all of the myeloid cell types were significantly reduced in workers exposed to benzene at exposure levels of  $<1$  p.p.m., and the largest reductions were seen in workers with exposure levels  $\geq 10$  p.p.m., including a 32% decline in granulocytes in these highest exposed workers compared with unexposed workers. In workers exposed to FA, reductions of about 10% ( $P = 0.02$ ) in granulocytes and 12% in platelets ( $P = 0.01$ ) were observed compared with unexposed workers (Figure 1A).

### Cells of lymphoid origin

The effects of these chemicals on lymphoid cells are shown in Figures 1 and 2. Total lymphocyte counts were significantly

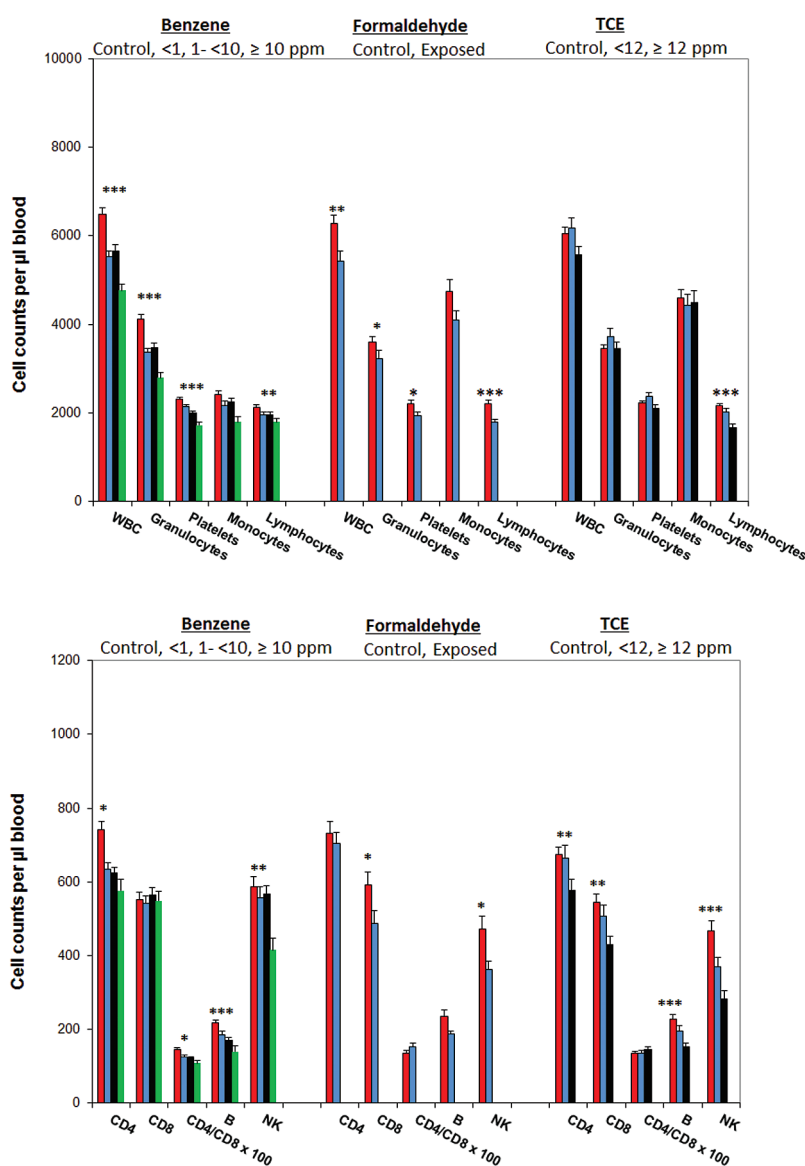


Figure 1. (A) Comparison of results from the benzene, FA and TCE exposure studies for peripheral blood cell counts (unadjusted means/cells  $\mu\text{l}$  and standard errors; monocyte counts multiplied by 10 and platelet counts divided by 100 for display purposes). All models adjusted for age, sex, current smoking, current alcohol consumption, BMI and/or recent infection. \* $P_{\text{trend}} < 0.05$ ; \*\* $P_{\text{trend}} < 0.01$ ; \*\*\* $P_{\text{trend}} < 0.001$ . (B) Comparison of results from the benzene, FA and TCE exposure studies for lymphocyte subsets (unadjusted means/cells  $\mu\text{l}$ ). All models adjusted for age, sex, current smoking, current alcohol consumption, BMI, and/or recent infection; \* $P_{\text{trend}} < 0.05$ ; \*\* $P_{\text{trend}} < 0.01$ ; \*\*\* $P_{\text{trend}} < 0.001$ .



decreased in exposed workers compared with unexposed workers for each of the three chemicals (Figure 1A), with significant exposure-dependent declines observed in workers exposed to benzene ( $P_{\text{trend}} < 0.01$ ) and TCE ( $P_{\text{trend}} < 0.001$ ). Reductions in total lymphocytes were apparent across all exposure levels for workers exposed to benzene compared with unexposed workers, including an 8% decline in workers exposed to <1 p.p.m. ( $P = 0.02$ ), whereas a significant decline associated with TCE exposure was limited to workers with the highest exposure level of  $\geq 12$  p.p.m. (22%;  $P < 0.0001$ ).

Examination of lymphocyte subsets revealed significant declines associated with each of the three chemicals for one or more cell types, with the strongest effects in terms of the number of affected cell types observed for workers exposed to benzene and TCE (Figure 1B). Benzene and TCE exposures were associated with significant exposure-dependent declines in CD4<sup>+</sup> T cells, B cells and NK cells, and a decline in CD8<sup>+</sup> T cells was apparent for workers exposed to TCE ( $P_{\text{trend}} < 0.01$ ). Significant reductions in lymphoid cell types associated with FA exposure were observed for CD8<sup>+</sup> T cells and NK cells (Figure 1B). Similar to the effects observed for myeloid cell types, significant declines were observed for benzene exposures <1 p.p.m. for B cells (15%), CD4<sup>+</sup> T cells (14%) and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio (14%), whereas significant reductions for TCE were limited to exposures  $\geq 12$  p.p.m. (Figure 1B).

The relationship between FA and TCE exposure and subsets of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells are shown in Figure 2. Exposure-dependent declines in CD4<sup>+</sup> naive T cells ( $P_{\text{trend}} = 0.02$ ), CD4<sup>+</sup> effector memory T cells ( $P_{\text{trend}} = 0.001$ ) and CD8<sup>+</sup> naive T cells ( $P_{\text{trend}} < 0.0001$ ) were observed in workers exposed to TCE compared with unexposed workers. A significant decline in CD8<sup>+</sup> effector memory T cells (20%,  $P = 0.02$ ; Figure 2) occurred in workers exposed to FA.

### Soluble CD27 and CD30 concentrations

Plasma levels of sCD27 declined by 63% in workers with TCE exposure levels <12 p.p.m., and by 60% in workers with exposure levels  $\geq 12$  p.p.m. compared with unexposed workers (Figure 3A). Levels of sCD30 were decreased by about 36% and 33% in the low and high TCE exposure categories ( $P < 0.01$ ), respectively. We further analyzed soluble levels of CD27 and CD30 in the benzene

and FA exposed workers, the results of which are shown in Figure 3A–B. Benzene exposure levels of  $\geq 10$  p.p.m. were associated with about a 17% reduction in soluble CD27 concentration compared with control workers, adjusted for age and sex ( $P = 0.03$ ). Further adjustment for other variables, including current alcohol use, current smoking, BMI and recent infection, resulted in similar findings ( $P = 0.02$ ). Benzene-exposed workers with exposure levels  $\geq 10$  p.p.m. were observed to have a similar magnitude of decline in levels of sCD30 as observed for sCD27, compared with unexposed workers, but this decline was not statistically significant when adjusting for age and sex ( $P = 0.16$ ; Figure 3B). Plasma concentrations of soluble CD27 were very similar and not significantly different in FA-exposed and unexposed workers ( $P = 0.63$ ; Figure 3A). Mean levels of soluble CD30 were reduced by about 14% in FA exposed workers compared with unexposed workers but this reduction was not statistically significant (Figure 3B).

### Monosomy 7 in CFU-GM cells

A comparison of the frequency of monosomy 7 in CFU-GM cells for the benzene and FA studies is shown in Figure 4A and B. A significant increase in the frequency of monosomy 7 was apparent in the cultured CFU-GM cells of workers exposed to FA ( $P < 0.0001$ ), and a significant exposure-dependent increase was observed in benzene-exposed workers ( $P_{\text{trend}} = 0.003$ ), compared with unexposed workers. Workers exposed to <10 p.p.m. of benzene had a 20% increase in levels of monosomy 7 ( $P = 0.04$ ), whereas a 48% increase in levels of monosomy 7 was observed in workers exposed to  $\geq 10$  p.p.m. of benzene ( $P = 0.005$ ), compared with unexposed workers.

### Discussion

Our findings from these cross-sectional biomarker studies in healthy, occupationally exposed workers revealed that WBC and lymphocyte counts, and one or more lymphocyte subsets, were significantly decreased in each of the groups of workers exposed to benzene, TCE and FA compared with unexposed workers. Benzene and FA exposure, but not TCE, were associated with declines in myeloid cell types, while reductions in lymphoid cell types were observed in workers exposed to all three chemicals to varying

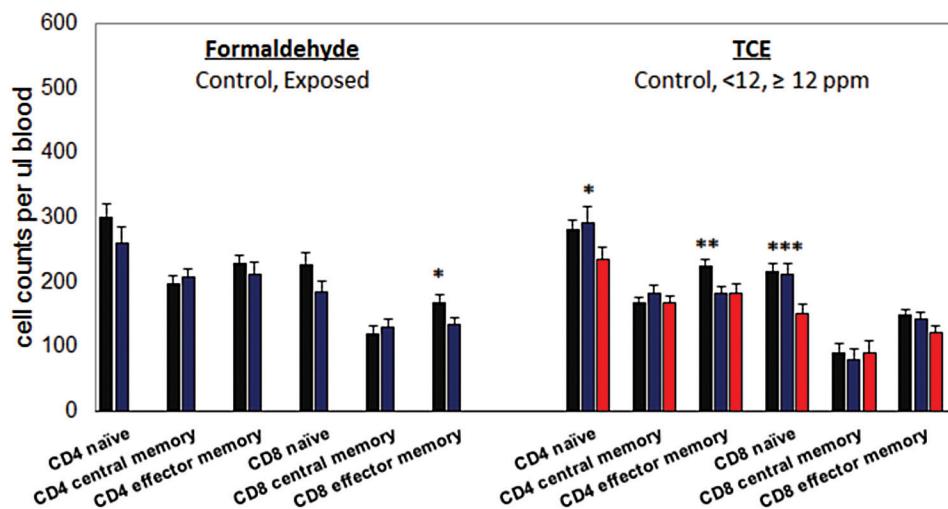
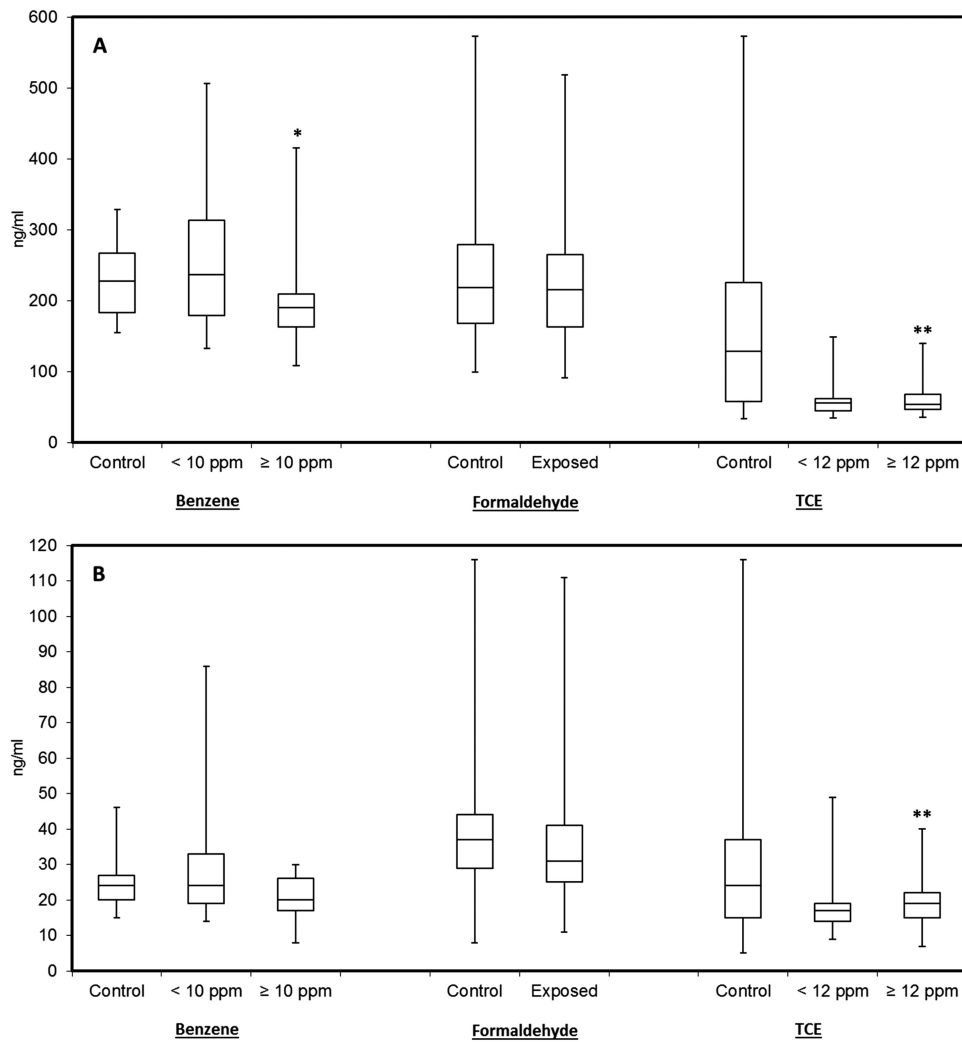
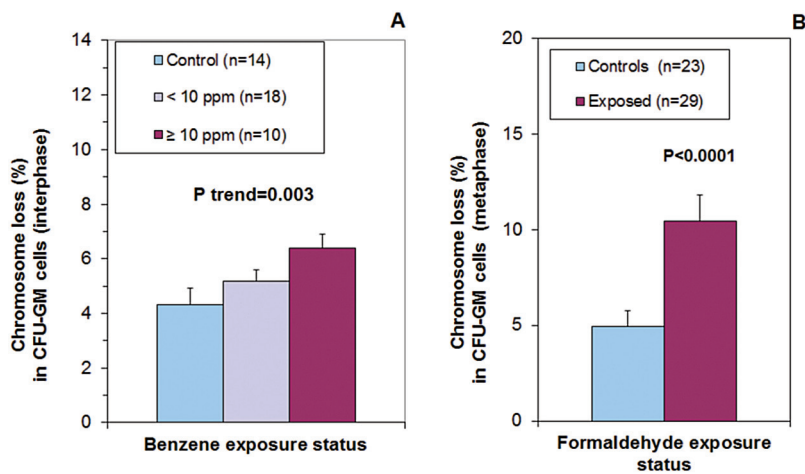


Figure 2. Comparison of results from the FA and TCE exposure studies for CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets (unadjusted means/cells  $\mu\text{l}$  and standard errors; CD8 central memory counts multiplied by 10). All models adjusted for age, sex, current smoking, current alcohol consumption, BMI and/or recent infection. \* $P_{\text{trend}} < 0.05$ ; \*\* $P_{\text{trend}} < 0.01$ ; \*\*\* $P_{\text{trend}} < 0.001$ .



**Figure 3.** Comparison of results from the benzene, FA and TCE exposure studies for soluble CD27 (A) and CD30 (B) concentrations. sCD27 and sCD30 data available from the entire study population for the TCE study (80 exposed, 96 control), and from a subset of the benzene (55 exposed, 29 control) and FA (43 exposed, 46 control) study populations. Box and whisker plots indicate median (line), interquartile range (box), and whiskers to the lowest and highest values in relation to benzene, FA and TCE exposures. (A) Final models for sCD27 adjusted for age and sex in the benzene study; age, sex, current alcohol consumption, BMI and recent infection in the FA study; and age, sex, and recent infection in the TCE study; \* $P_{\text{trend}} = 0.05$ ; \*\* $P_{\text{trend}} < 0.001$ . (B) Final models for sCD30 adjusted for age and sex in benzene study; age, sex, recent infection and BMI in the FA study; and age, sex and recent infection in the TCE study; \*\* $P_{\text{trend}} < 0.01$ .



**Figure 4.** (A) Mean frequency of monosomy 7 (with corresponding standard errors) in CFU-GM cells in workers exposed to benzene and control workers. Data available for 28 exposed subjects and 14 controls. (B) Mean frequency of monosomy 7 (with corresponding standard errors) in CFU-GM cells in workers exposed to FA and control workers. Data available for 29 exposed subjects and 23 controls.

degrees but were generally most apparent in workers exposed to benzene or TCE. We further showed that workers exposed to benzene or FA had elevated frequencies of monosomy 7 in cultured CFU-GM cells, a cytogenetic abnormality that is frequently associated with myeloid leukemia. Finally, examination of B-cell activation markers revealed a significant decrease in workers exposed to TCE for sCD27 and sCD30 and higher levels of benzene for sCD27. Considering the current epidemiologic evidence that suggests that each of these chemicals are associated with some LHMs but not others, comparisons of these hematotoxic effects across chemicals provide insight into their distinct patterns of toxicity and provide some mechanistic support for the chemical-specific associations with particular hematologic cancers.

Mature hematopoietic cells arise from one of two primary lineages, namely the myeloid that includes granulocytes, monocytes, thrombocytes, as well as erythrocytes and mast cells, and the lymphoid that includes T cells, B cells and NK cells. T cells, undergo further differentiation into CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the thymus, and these remain immunologically 'naive' until antigenic stimulation leads to further differentiation into effector cells that react with antigens at sites of inflammation (26). Whereas leukemias may involve both lymphoid and myeloid cells depending on the subtype, lymphomas result from the malignant transformation of B, T or NK cells that originate from the lymphoid lineage.

Myeloid leukemias, particularly AML, which has been associated with both benzene and FA exposures (4), are characterized by mutations that result in abnormal myeloblast cells in the bone marrow and blood that are capable of proliferation but not further differentiation into mature cells (27). We had previously reported that exposure to benzene and FA resulted in cell toxicity or genotoxicity to MPCs, which are close developmentally to stem cells from which myeloid leukemia originates (19). Decreases in MPCs cultured from peripheral blood may be a marker for toxicity to these cells in the bone marrow. Therefore, our findings showing reductions in cell types from the myeloid lineage in workers exposed to benzene or FA may reflect early biologic effects of these chemicals that are potentially relevant to the future development of myeloid leukemia, and provide biologic support to these associations in epidemiologic studies. This hypothesis is supported by our previous observations that demonstrated reductions in colony formation of MPCs in workers exposed to benzene or FA, as well as our data demonstrating increased levels of monosomy 7 in MPCs in workers exposed to either of these two chemicals (22,23). Notably, monosomy 7 is a frequent cytogenetic change in myeloid leukemia and myelodysplastic syndromes, and as such could be a plausible mechanism by which these chemicals contribute to myeloid leukemogenesis. It is notable that none of the peripheral blood cell counts from the myeloid lineage were significantly altered in TCE exposed workers, as absolute reductions in these cell types were <5% even in the highest exposed workers. The absence of an effect on myeloid-derived cell types for TCE is consistent with the lack of epidemiologic evidence for an association of TCE and myeloid leukemia (5).

Declines in cells of lymphoid origin and markers of lymphocyte activation, which were most pronounced in workers exposed to TCE or benzene in terms of the number of cell types affected, provide evidence that these chemicals can alter the immunologic profile of exposed workers, lending some plausibility for their suspected association with NHL. Immunosuppression, reflected in part as a decrease in CD4<sup>+</sup> T cells, is associated with an increased risk of NHL in several populations, including HIV infected individuals (28,29) and in organ transplant patients

(30,31). Further evaluation of specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets in the TCE study showed that higher exposed workers had declines ranging from ~16% to 29% for both CD4<sup>+</sup> and CD8<sup>+</sup> naive cells, and for CD4<sup>+</sup> and CD8<sup>+</sup> effector memory T cells, which is indicative of an impaired capacity to respond to antigenic-related inflammation and is notable given the established relationship between altered immunity and NHL (32). Our results suggest that both benzene and TCE result in cellular alterations, particularly a decline in CD4<sup>+</sup> T cells, that have been associated with the development of NHL in other disease contexts.

The soluble immune markers sCD27 and/or sCD30 were significantly lower in subjects exposed to TCE and higher levels of benzene, but not FA. Both markers are reflective of B-cell activation and have immunoregulatory activities, including regulation of the Th1/Th2 balance. Recent studies have demonstrated that higher levels of sCD27 and sCD30 are associated with increased NHL risk in general population-based prospective studies (24). In contrast, we observed a lower level of these markers among workers exposed to TCE and higher levels of benzene. The subjects in our study are, however, in a special situation in that they are exposed to toxins in the workplace that are causing subtle hematotoxicity and possibly immunosuppression in a manner that is usually not present, or cannot be identified, in general population cohort studies. As such, it is possible that a lower level of these markers among workers exposed to hematotoxins could put them at increased future risk of NHL as well, but through alternative mechanisms (e.g. development of a B-cell clone that escapes from the immunosuppressive effects of these exposures). Evaluation of this hypothesis would require prospective monitoring studies of specific populations of workers exposed to these chemicals.

Total lymphocytes and some lymphocyte subsets were also reduced in workers exposed to FA, providing evidence that FA exposure can also result in alterations in cell types that are associated with immune suppression. These findings are notable given the increasing evidence that immune suppression may also be related to the risk of myeloid leukemia, which has been linked to FA exposure in some epidemiologic studies (4); for example, risk of AML was significantly elevated in a recent large registry-based study of over 200,000 solid organ transplant recipients (33). However, in the context of NHL risk, which has not been elevated among FA exposed populations, it is notable that FA exposure was demonstrated to have minimal effect on overall CD4<sup>+</sup> T-cell counts (i.e. 3.8% reduction in exposed workers) and the extent to which other lymphoid-derived cell types were reduced in these workers was generally less apparent as compared with workers exposed to higher levels of benzene and TCE. In the setting of HIV, a lower CD4 count has been established as an important predictor of future NHL risk, with clear exposure-response patterns observed suggesting that the magnitude of CD4 decline in this setting is an important determinant of risk (34). To our knowledge, no epidemiologic study has prospectively evaluated whether alterations in specific lymphocyte subsets are associated with future NHL risk in healthy individuals in the general population. Such studies may be particularly useful in evaluating the relative importance of specific subsets in relation to NHL risk, and would provide potentially important insight into the interpretation of the chemical-specific hematotoxic patterns observed in our studies with respect to the implications for future hematologic cancer risks.

The hematotoxicity of these chemicals, particularly for benzene and FA, has been investigated in other study populations. For FA, several studies in China have observed lower WBC counts in workers exposed to FA levels as low as 0.02–0.04 mg/m<sup>3</sup> (35),



and significant alterations in platelets and lymphocyte subsets have also been reported in FA exposed workers although these findings have been less consistent (35–38). The hematotoxicity of benzene exposure has been well-described as chronic exposure is associated with the development of various blood dyscrasias including aplastic anemia and myelodysplastic syndrome, even at relatively low levels of exposure (39). Relatively consistent associations with lower levels of WBCs, red blood cells (RBCs), lymphocytes, and platelets have been reported in workers exposed to high levels of benzene (40,41); however, demonstration of these effects at lower levels of exposure (e.g. <1 ppm) has been less consistent. For example, other studies in China of workers employed in shoe factories have observed significant declines in WBCs or RBCs at these lower levels of exposure (42,43) but the findings are in contrast with studies conducted in Western countries that have generally not observed hematotoxic effects at lower levels of exposure based on data obtained from routine monitoring of exposed workers (44–46). Additional occupational studies of these chemicals would be informative to further evaluate associations with hematologic parameters, particularly for TCE given the limited available data in human populations.

In summary, our study compared and contrasted the hematological effects of occupational exposure to benzene, FA and TCE. Declines in myeloid derived cells were associated with benzene and FA, but not TCE exposure, while all three chemicals were associated with declines in WBC counts and total lymphocyte counts, as well as several lymphocyte subsets. The decline in lymphoid cell types was most pronounced in workers exposed to the highest levels of TCE and benzene, and statistically significant reductions in sCD27 and/or sCD30 were limited to these two chemicals. Given that alterations in myeloid and lymphoid cell types have been associated with hematological malignancies, our data provide biologic insight into the epidemiological evidence linking exposure to benzene and FA with risk of myeloid leukemia, and TCE and benzene with risk of lymphoid malignancies.

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