

Occupational Exposure to Trichloroethylene is Associated with a Decline in Lymphocyte Subsets and Soluble CD27 and CD30 Markers

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Abstract

Occupational cohort and case-control studies suggest that TCE exposure may be associated with non-Hodgkin lymphoma (NHL) but findings are not consistent. There is a need for mechanistic studies to evaluate the biologic plausibility of this association. We carried out a cross-sectional molecular epidemiology study of 80 healthy workers that used TCE and 96 comparable unexposed controls from clothes manufacturing and food processing factories in Guangdong, China. Personal exposure measurements were taken over a three-week period before blood collection. Ninety-six percent of workers were exposed to TCE below the current US OSHA Permissible Exposure Limit (100 ppm 8h TWA), with a mean (SD) of 22.2 (36.0) ppm. The total lymphocyte count and each of the major lymphocyte subsets including CD4+ T cells, CD8+ T cells, natural killer (NK) cells, and B cells were significantly decreased among the TCE-exposed workers compared to controls ($p < 0.05$), with evidence of a dose-dependent decline. Further, there was a striking 61% decline in sCD27 plasma level and a 34% decline in sCD30 plasma level among TCE exposed workers compared to controls. This is the first report that TCE exposure under the current OSHA workplace standard is associated with a decline in all major lymphocyte subsets and sCD27 and sCD30, which play an important role in regulating cellular activity in subsets of T, B, and NK cells and are associated with lymphocyte activation. Given that altered immunity is an established risk factor for NHL, these results add to the biologic plausibility that TCE is a possible lymphomagen.

Introduction

Trichloroethylene (TCE) is a volatile chlorinated organic compound and commonly used in industrial settings as a degreaser for metal parts and general-purpose solvent for lipophilic compounds. In 1995 it was estimated that more than 400,000 workers were exposed to TCE on an annual basis in the United States [1]. Further, as a consequence of its presence in workplaces for many years, TCE has become a widespread environmental water contaminant.

Approximately 34% of the drinking water supplies that have been tested in the United States had some level of TCE contamination, and it was reported that about 10% of the United States population had detectable levels of TCE in their blood [2,3].

TCE is a rodent carcinogen [4] but its carcinogenicity in humans is unclear. There is a voluminous experimental literature on short-term biologic effects of TCE exposure and recent studies indicate that TCE alters key lymphocyte subset levels, particularly CD4⁺ T cell populations, and inhibits activation-induced cell death of CD4⁺ T cells [5-7]. There is some evidence of an association with non-Hodgkin lymphoma (NHL) in epidemiological studies but findings are not entirely consistent [4,8-11]. The 1995 IARC review of TCE classified TCE as a probable (Group 2A) human carcinogen based on sufficient evidence of TCE's carcinogenicity in animals and limited evidence of TCE's carcinogenicity in humans, with elevated risks suggested for liver and biliary tract cancer and NHL [4,11]. However, the carcinogenicity of TCE and its regulation is a matter of continuing debate despite an extensive database of *in vitro* and *in vivo* animal studies and several cohort and case-control studies.

Wartenberg et al., noted that few epidemiologic studies of TCE carcinogenicity have been able to evaluate the isolated effects of TCE and recommended that biomarker studies of

populations with isolated exposure to TCE be carried out [11]. The few published cross-sectional studies of workers are relatively small and have serious limitations with regard to evaluation of potential confounding exposures. To address questions about TCE's potential mechanism of action in humans, we carried out a cross-sectional study to evaluate the impact of occupational exposure to TCE on peripheral blood cells including lymphocyte subset counts and soluble (s) CD27 and sCD30, two members of the tumour necrosis factor receptor superfamily that play an important role in regulating cellular activity in subsets of T, B, and natural killer (NK) cells [12-14].

METHODS

Study Design

To select factories for study, we conducted an initial screening of more than 40 potential study factories over a one-year period using Dräger tubes and 3M badges to measure TCE and other chemicals including benzene, styrene, ethylene oxide, formaldehyde, methylene chloride, chloroform, perchloroethylene and epichlorohydrin. Factories were included if they used TCE in manufacturing processes, had no detectable benzene, styrene, ethylene oxide, formaldehyde, or epichlorohydrin levels, and low to negligible levels of other chlorinated solvents. Duplicate badges for TCE were analyzed in Guangdong and the United States and showed similar results with a Pearson correlation of 0.99. Ultimately, six study factories with metal (n=4), optical lenses (n=1) and circuit boards (n=1) cleaning processes were identified that fulfilled the above selection criteria.

In June and July, 2006, we carried out a cross-sectional study of 80 workers currently exposed to TCE in the six study factories with TCE cleaning operations and 96 unexposed controls. Control subjects were enrolled from two clothes manufacturing factories, one food production factory, and a hospital that did not use TCE and were in the same geographic region as the factories that used TCE. Controls were frequency-matched by sex and age (± 5 years) to exposed workers. Exclusion criteria for both TCE-exposed and control workers were history of cancer, chemotherapy, and radiotherapy, as well as previous occupations with notable exposure to benzene, butadiene, styrene and/or ionizing radiation. The study was approved by Institutional Review Boards at the U.S. National Cancer Institute and the Guangdong National Poison Control Center, China. Participation was voluntary and all subjects gave written informed consent.

Exposure Measurement and Sample Collection

Full-shift personal air exposure measurements, two-to-three per subject, were taken in a three-week time-period in the factories using 3M organic vapor monitoring (OVM) badges before blood-collection. All samples were analyzed for TCE and a subset (48 from TCE-exposed workers) was analyzed for a panel of organic hydrocarbons including benzene, methylene chloride, perchloroethylene and epichlorohydrin. In two of the metal degreasing factories workers were intermittently using respirators. Only one subject wore gloves while cleaning with TCE. OVM samples were obtained on a subgroup of control workers in the food and clothes production factories. Subjects were interviewed using a questionnaire that requested information about demographic and lifestyle characteristics and occupational history. They were also asked to provide a 29 ml peripheral blood sample, buccal cell mouth rinse sample, post-shift and overnight urine samples, and undergo a brief physical exam that included measurement of blood

pressure, height, weight and temperature, and evaluation for signs of current upper or lower respiratory infection.

Assays

Blood samples were delivered to the laboratory within six hours of being collected, where the Complete Blood Count (CBC) and differential and major lymphocyte subsets were analyzed on the same day that a peripheral blood sample was collected. Plasma sCD27 and sCD30 were measured in duplicate by an enzyme-linked immunosorbent assay (Bender Medsystems). Exposed workers and their matched controls were assayed consecutively within the same batch. Measurements from blinded quality control replicates interspersed among the samples did not identify outlier batches. Assay CVs were < 10% for each parameter from the CBC and lymphocyte subset analysis and were 31% and 32% for sCD27 and sCD30, respectively. Normal ranges for peripheral blood cells are listed in Supplementary Table 1.

Statistical Analysis

Unadjusted summary measures are presented for all endpoints. Linear regression using the natural logarithm (ln) of each endpoint was used to test for differences between control and exposed workers, and to evaluate for a dose-response across exposure groups, i.e., controls, workers exposed to <12 ppm TCE (the median exposure level), and \geq 12 ppm TCE. TCE air levels were based on the arithmetic mean of an average of two to three measurements per subject. All statistical models included the frequency-matching factors age (as a continuous variable) and sex. In addition, potential confounders that have been previously shown to influence one or more of the endpoints in this report were included in a model for a given

endpoint if the regression coefficient was altered by $\pm 15\%$, and included current cigarette smoking status (yes/no), current alcohol consumption (yes/no), recent infections (flu or respiratory infections in the previous month), and body mass index (BMI). The total lymphocyte percent from the CBC was used to calculate three lymphocyte subsets (i.e., CD4⁺ T cells; CD8⁺ T cells, NK cells), and an additional calculation was carried out using the lymphocyte percent obtained by flow cytometry and compared for quality control purposes. If there was more than a 15% standard deviation between the two measures, then the samples were deleted for the analysis of lymphocyte subsets (i.e., three samples, 1.7% of data). All analyses were carried out using SAS version 9.0 software (SAS Institute, Cary, North Carolina, USA).

Results

Subjects were categorized into three groups by mean TCE levels measured during the month before phlebotomy [controls, <12 ppm (mean = 5 ppm), and ≥ 12 ppm (mean = 38 ppm)]. Demographic characteristics including age, sex distribution, current smoking status, and BMI were comparable among the three groups (Table 1). TCE exposure was negligible in the control factories.

The total lymphocyte count and all major lymphocyte subsets including CD4⁺ T cells; CD8⁺ T cells, NK cells, and B cells, were statistically significantly lower in workers exposed to TCE than controls, and there was a significant dose-response relationship across the categories of controls, and lower and higher exposed workers (Figure 1a and Figure 1b, Supplementary Table 2). In contrast, there was no evidence that TCE exposure influenced the granulocyte, platelet, or monocyte counts (Figure 1a, Supplementary Table 2). There was a small decline in plasma

hemoglobin concentration but no difference in the red blood cell mean corpuscular volume or platelet counts (Supplementary Table 2). There was a substantial decline in sCD30 and a striking decline in sCD27 (i.e., 61%) plasma levels in TCE-exposed individuals compared to controls (Figure 2), and workers exposed to even relatively low levels of TCE (< 12 ppm) had significantly lower levels of both compounds (Supplementary Table 2).

To evaluate the influence of exposure to other chlorinated solvents that were present at relatively low levels in some factories, we excluded one or more of these factories at a time from the analyses, and found that results were similar and conclusions unchanged (data not shown). We repeated the analyses in the 77 (out of a total of 80) workers exposed to under 100 ppm TCE, the current Occupational Safety and Health Administration Permissible Exposure Limit (PEL) (http://www.osha.gov/dts/chemicalsampling/data/CH_273000.html), and found that the strength of the associations shown in Figures 1a, 1b, and 2 were essentially unchanged. Further, we carried out analyses among the 60 workers exposed to less than 25 ppm TCE, which is the current National Institute of Occupational Safety and Health (NIOSH) Recommended Exposure Limit (REL), and found that the total lymphocyte count, all lymphocyte subsets, and sCD27 and sCD30 concentrations were still significantly lower at $p < 0.05$ compared to controls.

Discussion

We found that exposure to relatively low levels of TCE, including at levels lower than the OSHA PEL and NIOSH REL, was associated with a dose-dependent decline in total peripheral blood lymphocytes and all major lymphocyte subsets, including CD4⁺ T cells, which is consistent with

experimental reports [6,7]. Further, we showed that TCE exposure was also associated with a decline in sCD27 and sCD30, and that sCD27 levels appeared to be particularly sensitive to TCE exposure.

TCE exposure has been associated with immunosuppression in animals [6,15]. In humans, there are little data on immunosuppression from TCE exposure [6,11,16]. There is some evidence that exposure to TCE in humans is associated with alterations in levels of certain cytokines [12-14,17]. There is also some evidence that TCE exposure is associated with several autoimmune diseases in humans such as systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and dermatitis [6,11]. Our data show that TCE exposure was associated with decline in all major lymphocyte subsets tested and with important markers of lymphocyte function and activation, sCD27 and sCD30, which can play a key role in maintaining control of T-cell regulation [12-14]. The fact that we see strong effects on lymphocytic parameters and no effect on granulocytes, platelets or monocytes suggests that there is a relatively selective effect on lymphoid progenitors or on the lymphocyte maturation process, rather than a broad impact on stem cells in the bone marrow. These results contrast with the pattern we and others have observed among humans exposed to benzene, i.e., a strong and broad effect across all blood cell types that likely results from suppression of bone marrow stem or progenitor cell development [18].

Both sCD27 and sCD30 shed by B and T cells at activation are important co-stimulator molecules in the regulation of the balance between Th1 and Th2 responses [12,14]. They have been found to be altered in patients with a variety of immunopathological diseases and have been used as markers to monitor immune activation as well as to assess therapeutic effects of immunosuppressive and antiviral treatment [19-22]. There is a possibility that the effects we see

for sCD27 and sCD30 and TCE exposure could simply be a function of suppressed lymphocyte counts. However, we did not observe a correlation between lymphocyte subsets and sCD27 and sCD30 in TCE-exposed workers (not shown), consistent with other reports [23].

We observed a decrease in lymphocyte subsets and a substantial decline in markers of lymphocyte activation. Given that altered immunity including immunosuppression is an established risk factor for NHL, these results add to the biologic plausibility that TCE is a lymphomagen [24]. More specifically, a decrease in CD4⁺ T cells has been associated with an increased future risk of NHL in HIV infected populations [25], in post-renal transplantation patients who receive immunosuppressive therapy [26], and in patients with Sjogren's syndrome [27]. As noted, both sCD27 and sCD30 are considered markers of lymphocyte activation. Decreased levels of sCD27 following chemotherapy and bone-marrow transplantation have been reported, consistent with an immunosuppressive state induced by certain regimens [28,29].

Our study is the first investigation of the impact that TCE exposure has on lymphocyte subsets, sCD27 and sCD30. We report that TCE was associated with a decrease in all major lymphocyte subsets (i.e. CD4⁺ T cells; CD8⁺ T cells, NK cells, and B cells) and soluble CD27 and CD30 among workers exposed to TCE under the current OSHA workplace standard. These results, if confirmed in future studies, show that TCE exposure broadly affects several components of the immune system and provide additional support for the biologic plausibility that TCE is associated with risk of NHL.

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Ref Type: Thesis/Dissertation
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Table 1. Demographic characteristics and trichloroethylene (TCE) exposure level

Subjects	Controls (n=96)	Exposed		
		Total (n=80)	<12 ppm (n=39)	≥ 12 ppm (n=41)
Demographic Characteristics				
Age, mean (SD) ¹	27 (7)	25 (7)	24 (5)	27 (8)
BMI, mean (SD) ¹	22 (3)	21 (3)	21 (2)	22 (3)
Sex ²				
Female n (%)	23 (24)	23 (29)	15 (38)	8 (20)
Male n (%)	73 (76)	57 (71)	24 (62)	33 (80)
Current smoke ²				
No n (%)	58 (60)	46 (58)	22 (56)	24 (59)
Yes n (%)	38 (40)	34 (42)	17 (44)	17 (41)
Current alcohol use ²				
No n (%)	56 (58)	54 (68)	26 (67)	28 (68)
Yes n (%)	40 (42)	26 (32)	13 (33)	13 (32)
Recent infection ²				
No n (%)	75 (78)	65 (81)	31 (79)	34 (83)
Yes n (%)	21 (22)	15 (19)	8 (21)	7 (17)
TCE Exposure				
TCE air level (ppm) ³				
Mean (SD)	<0.03	22.19 (35.94)	5.19 (3.47)	38.36 (44.61)

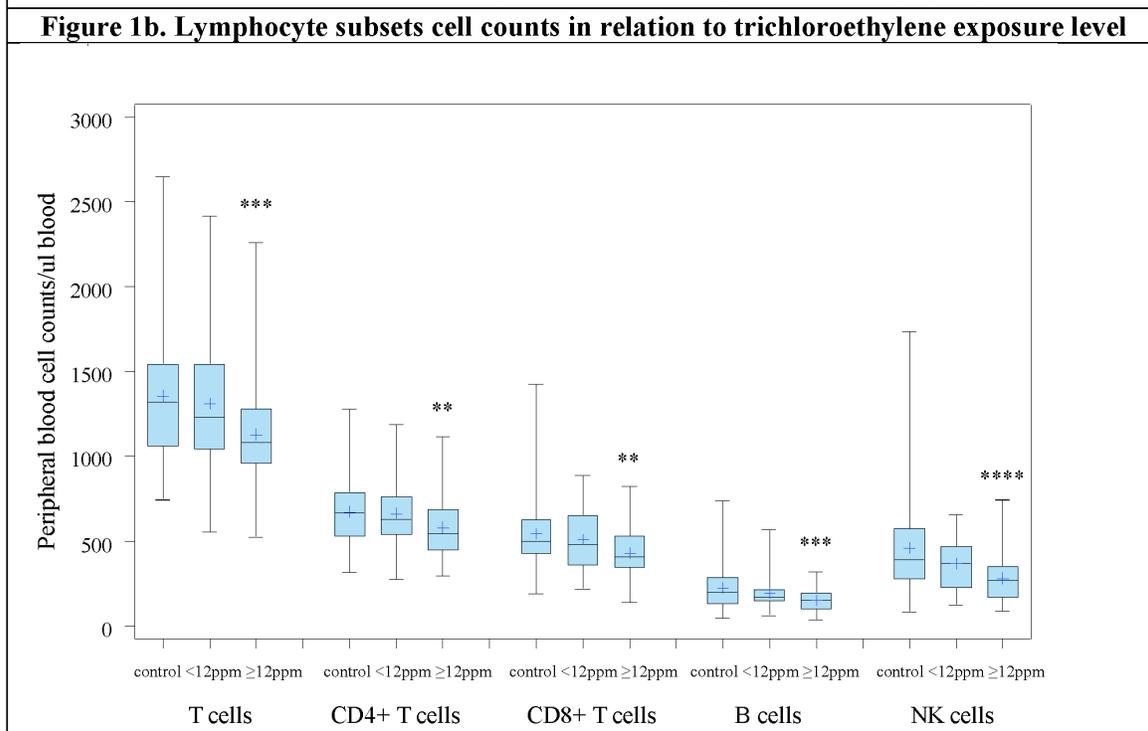
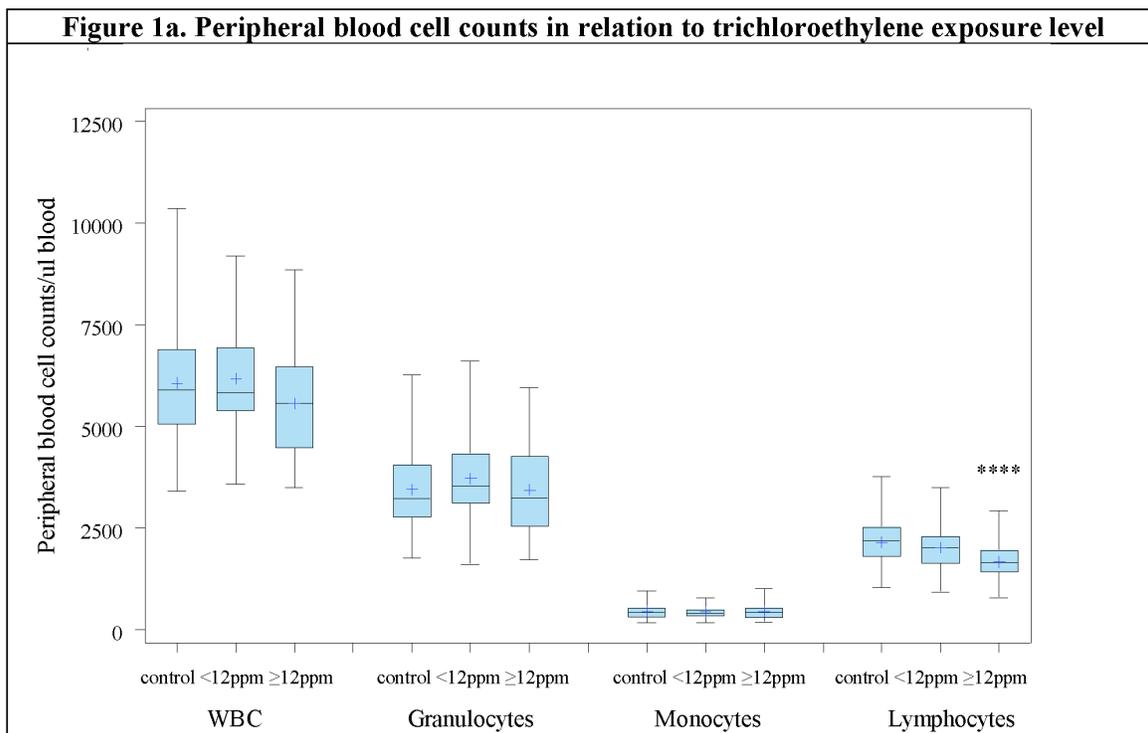
¹ Mean ± standard deviation.² Number (percent).³ TCE air level is the arithmetic mean (±SD) of an average of two measurements per subject collected during the month before phlebotomy.

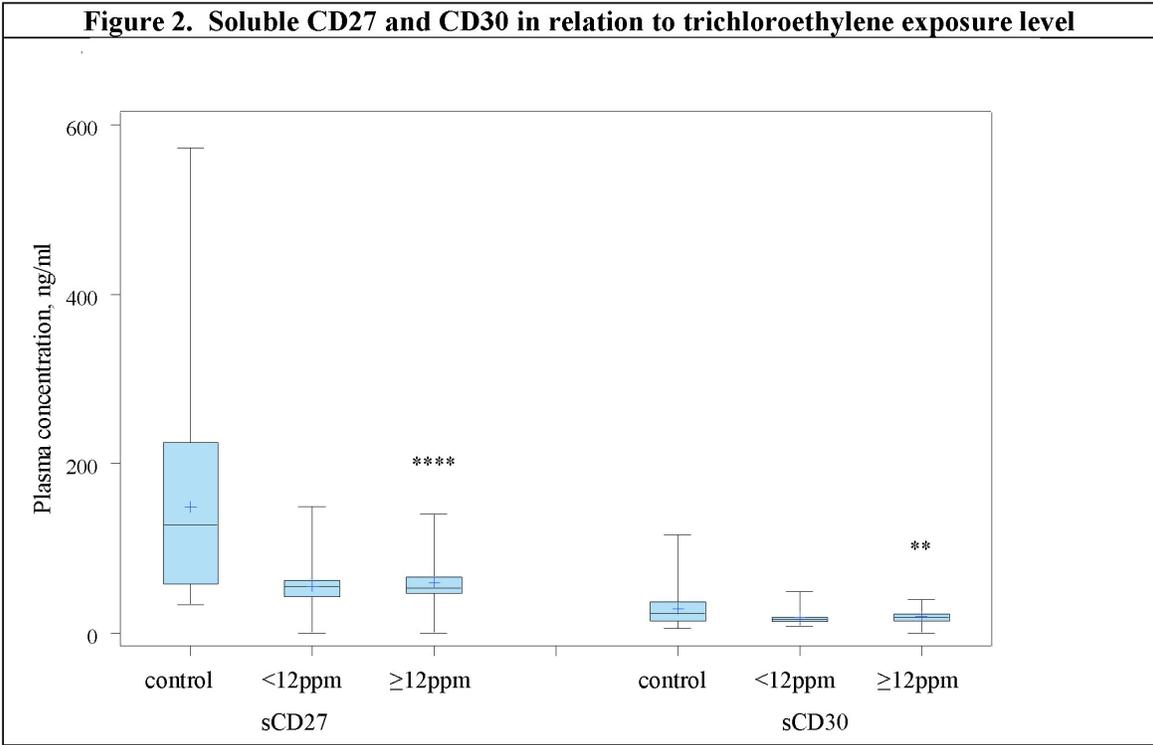
FIGURE LEGENDS

Figure 1a. Peripheral blood cell counts in relation to trichloroethylene exposure level. P_{trend} using category of trichloroethylene levels (controls, <12 ppm, \geq 12 ppm) as a continuous variable. 12 ppm was the median trichloroethylene concentration of the exposed subjects. Differences in cell counts were tested by linear regression analysis of ln transformed endpoint, adjusting for relevant covariates (WBC: adjusted for age, sex, smoking status, and BMI; Granulocytes: adjusted for age, sex, and BMI; Monocytes: adjusted for age, sex, and smoking status; Lymphocyte: adjusted for age and sex). The p values are indicated as: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$; **** $p < 0.0001$.

Figure 1b. Lymphocyte subsets cell counts in relation to trichloroethylene exposure level. Differences in cell counts were tested by linear regression analysis of ln transformed endpoint, adjusting for relevant covariates (CD4⁺, CD8⁺, and NK T cells: adjusted for age and sex, three subjects (2 controls and 1 exposed) were deleted due to inconsistent cell counts using CBC data vs. flow cytometry to calculate % lymphocytes; B cell: adjusted for age, sex, and smoking status).

Figure 2. Soluble CD27 and CD30 in relation to trichloroethylene exposure level. P_{trend} using category of trichloroethylene levels (controls, <12 ppm, \geq 12 ppm) as a continuous variable. 12 ppm was the median trichloroethylene concentration of the exposed subjects. Differences in sCD27 and sCD30 were tested by linear regression analysis of ln transformed endpoint, adjusting for age, sex, and infection. For sCD27, results available for 38 subjects exposed to less than 12 ppm TCE. The p values are indicated as: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$; **** $p < 0.0001$.





Supplementary Table 1. Normal ranges for peripheral blood cell counts and related parameters

	Normal range	Unit
Peripheral blood cell counts based on the CBC¹		
White blood cells	4000-10,000	cells/ μ l
Platelets	100,000-361,500	cells/ μ l
Monocytes	0-800	cells/ μ l
Hemoglobin	11.0-17.0	g /dl
Lymphocytes	800-6000	cells/ μ l
Lymphocyte subsets²		
CD4+ T cells	390–1,634	cells/ μ l
CD8+ T cells	283–881	cells/ μ l
CD4/CD8 Ratio	0.60–3.29	

¹ Normal range from Guangdong Pinioning Control Center laboratory

² Jiang, W., Kang, L., Lu, H. Z., Pan, X., Lin, Q., Pan, Q., Xue, Y., Weng, X., and Tang, Y. W. (2004). Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. Clin. Diagn. Lab Immunol. 11, 811-813.

Supplementary Table 2. Peripheral blood cell counts and sCD27 and sCD30 levels in relation to trichloroethylene (TCE) exposure level

Subject category	Controls (n=96)	Exposed (n=80)	<12 ppm (n=39)	≥ 12 ppm (n=41)	P for exposed vs. control	P _{trend} ¹	P for <12 ppm vs. control	P for ≥12 vs. control
Peripheral blood cell counts ²								
White blood cells ³	6060 (1348)	5864 (1364)	6177 (1386)	5566 (1288)	0.25	0.060	0.70	0.03
Granulocytes ⁴	3448 (945)	3581 (1066)	3724 (1100)	3445 (1029)	0.44	0.87	0.14	0.89
Platelets ⁵	222 (57.1)*10 ³	224 (49.9)*10 ³	237 (49.8)*10 ³	211 (47.2)*10 ³	0.88	0.62	0.26	0.43
Monocytes ⁶	459 (179)	446 (162)	442 (157)	450 (168)	0.53	0.64	0.51	0.72
Hemoglobin (g/dl) ⁷	14.29 (1.46)	13.73 (1.36)	13.53 (1.32)	13.92 (1.4)	0.0022	0.0066	0.01	0.02
Red blood cell mean corpuscular volume (fl) ⁸	87.60 (7.71)	89.32 (6.23)	90.11 (4.67)	88.57 (7.40)	0.17	0.36	0.11	0.52
Lymphocytes ⁵	2154 (552)	1837 (522)	2012 (546)	1671 (445)	0.0001	<0.0001	0.20	<0.0001
T cells ⁵	1356 (374)	1215 (378)	1310 (391)	1124 (346)	0.0039	0.0005	0.32	0.0004
CD4 ⁺ T cells ^{5,9}	675 (200)	620 (209)	664 (220)	577 (192)	0.033	0.0089	0.49	0.007
CD8 ⁺ T cells ^{5,9}	544 (216)	468 (167)	508 (175)	430 (150)	0.0092	0.0014	0.39	0.001
CD4/CD8 Ratio ⁵	1.36 (0.52)	1.41 (0.40)	1.36 (0.35)	1.45 (0.45)	0.22	0.15	0.65	0.14
B cells ⁶	227 (133)	172 (86.6)	194 (99.0)	152 (68.1)	0.0014	0.0002	0.19	0.0002
NK cells ^{5,9}	467 (279)	325 (152)	370 (148)	282 (145)	0.0003	<0.0001	0.21	<0.0001
Plasma concentration, ng/ml ¹⁰								
sCD27	148.79 (107.5)	57.47 (23.16)	55.30 (21.29) ¹¹	59.47 (24.85)	<0.0001	<0.0001	<0.0001	<0.0001
sCD30	28.68 (18.8)	18.88 (7.63)	18.45 (7.52)	19.28 (7.81)	0.0003	0.0012	0.0025	0.0041

¹ P_{trend} using category of trichloroethylene levels (controls, <12 ppm, ≥12 ppm) as a continuous variable. 12 ppm was the median trichloroethylene concentration of the exposed subjects.

² unadjusted mean (±SD) cells/μl blood.

³ adjusted for age, sex, smoking status, and BMI in regression analysis of ln transformed endpoint.

⁴ adjusted for age, sex, and BMI in regression analysis of ln transformed endpoint.

⁵ adjusted for age and sex in regression analysis of ln transformed endpoint.

⁶ adjusted for age, sex, and smoking status in regression analysis of ln transformed endpoint.

⁷ adjusted for age and sex, infection, BMI.

⁸ adjusted for age and sex.

⁹ three subjects (2 controls and 1 exposed) were deleted due to inconsistent cell counts using CBC data vs. flow cytometry to calculate % lymphocytes.

¹⁰ adjusted for age, sex, and infection in regression analysis of ln transformed endpoint.

¹¹ results available for 38 subjects exposed to less than 12 ppm TCE.