

Hematotoxicity Among Chinese Workers Heavily Exposed to Benzene

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Benzene is a well-established hematotoxin. However, reports of its effects on specific blood cells have been somewhat inconsistent and the relative toxicity of benzene metabolites on peripheral blood cells in humans has not been evaluated. We compared hematologic outcomes in a cross-sectional study of 44 workers heavily exposed to benzene (median: 31 parts per million [ppm] as an 8-hr time-weighted average [TWA] and 44 age and gender-matched unexposed controls from Shanghai, China. All hematologic parameters (total white blood cells [WBC], absolute lymphocyte count, platelets, red blood cells, and hematocrit) were decreased among exposed workers compared to controls, with the exception of the red blood cell mean corpuscular volume (MCV), which was higher among exposed subjects. In a subgroup of workers who were not exposed to more than 31 ppm benzene on any of 5 sampling days ($n = 11$, median 8 hr TWA = 7.6 ppm, range = 1–20 ppm), only the absolute lymphocyte count was significantly different between exposed workers (mean [sd] $1.6 [0.4] \times 10^3 \mu\text{L}$) and controls ($1.9 [0.4] \times 10^3 \mu\text{L}$, $p = 0.03$). Among exposed subjects, a dose-response relationship with various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) was present only for the total WBC count, the absolute lymphocyte count, and the MCV. Correlations between benzene metabolites and hematologic parameters were generally similar, although hydroquinone was somewhat more strongly associated with a decrease in the absolute lymphocyte count, and catechol was more strongly associated with an increase in MCV. Morphologic review of peripheral blood slides demonstrated an excess of red blood cell abnormalities (i.e., stomatocytes and target cells) only in the most heavily exposed workers, with no differences in granulocyte, lymphocyte, or platelet morphology noted. Although benzene can affect all the major peripheral blood elements, our results support the use of the absolute lymphocyte count as the most sensitive indicator of benzene-induced hematotoxicity. © 1995 Wiley-Liss, Inc.*

KEY WORDS: benzene, hematotoxicity, phenol, muconic acid, hydroquinone, catechol

Abbreviations: GC/MS, gas chromatography/mass spectroscopy; WBC, white blood cell; MCV, mean corpuscular volume; TWA, time-weighted average; ppm, parts per million; SHAC, Shanghai Hygiene and Anti-Epidemic Institute; NCI, National Cancer Institute; RBC, red blood cell.

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INTRODUCTION

Benzene has been known as a hematotoxin for more than a century [reviewed in Aksoy, 1988; Goldstein, 1977]. Although benzene has been shown to cause pancytopenia and aplastic anemia, the relative sensitivity of specific peripheral blood cells to benzene, in the absence of severe marrow toxicity, is uncertain. Discrepancies in the older literature may have been due to variation in the method of measuring blood cells, exposure to additional compounds such as toluene [Andrews et al., 1977] which may affect benzene metabolism, or failure to adjust for potential confounders.

As part of a large cohort study of cancer and hematotoxicity among approximately 75,000 workers exposed to benzene sponsored by The Chinese Academy of Preventive Medicine and the U.S. National Cancer Institute [NCI; Yin et al., 1995], we performed a cross-sectional study of currently exposed workers in order to gain insight into early biologic effects associated with benzene exposure. Here, we report the association between benzene exposure, benzene metabolite formation, and hematotoxicity.

MATERIALS AND METHODS

Factory Identification and Subject Enrollment

Workplaces were identified from public health district records and from exposure and demographic data previously collected for the cohort study [Yin et al., 1995]. Exposed factories were selected so that the study population would have a wide range of exposures to benzene (1 to ≥ 25 parts per million [ppm] as an 8-hr time-weighted average [TWA], similar to that found among workers in the cohort study), minimal exposure to toluene, and no other exposures to known hematotoxic or genotoxic agents. Factory 1 used benzene to solubilize natural rubber for subsequent production of rubber padding for printing presses. Factory 2 used benzene to manufacture adhesive tape. In Factory 3, benzene-based paint and varnish were applied to wooden toys and boxes. Two workplaces in the same geographic area that did not use benzene or other chemicals associated with bone marrow toxicity were selected as control factories. One factory manufactured sewing machines while the second workplace was an administrative facility.

Study subjects were enrolled in the fall of 1992 in Shanghai, China. Exclusion criteria for all study subjects included prior history of cancer, therapeutic radiation, chemotherapy, or current pregnancy. Eligibility criteria for exposed subjects were current employment for at least 6 months in a factory which used benzene, minimal exposure to other aromatic solvents, and no exposure to other known marrow-toxic chemicals or ionizing radiation. Eligibility

criteria for controls were no history of occupational exposure to benzene, other marrow-toxic chemicals, or ionizing radiation. Unexposed controls were frequency-matched to the exposed subjects on age (5-year intervals) and gender.

Industrial Hygiene Assessment

Current exposure assessment

Individual exposure was monitored by organic vapor passive dosimetry badges (3M no. 3500, St. Paul, Minnesota), which were worn by each worker for a full workshift on 5 separate days during the 1–2-week period prior to phlebotomy. Badges were analyzed at the Shanghai Hygiene and Anti-Epidemic Institute (SHAC) by gas chromatography (GC) with flame ionization detection for benzene, toluene, and xylene. An 8-hr TWA exposure was calculated for each compound as the geometric mean of the five air measurements. Only trace amounts of other aromatic solvents were detected in analyses. As a quality control check, two badges were worn for a full workshift by each of 10 workers who were exposed to a wide range of benzene levels. One badge was analyzed by the SHAC and the duplicate badge was analyzed by Clayton Environmental Consultants (Novi, Michigan). The 10 duplicate measurements were essentially identical (Spearman $r = 0.98$, $p < 0.0001$).

A detailed assessment of factory records showed that no other known marrow-toxic chemicals or physical agents were present in these workplaces. Sixteen workers in Factory 1 used half-mask charcoal-activated respirators during the highest exposure period of their workshift, which lasted about 3 hr per shift. Neither quantitative nor qualitative fit testing was performed. Workers typically changed the charcoal once every 1–2 days or more frequently, depending upon when they smelled benzene inside the respirator. In a preliminary analysis, factory type and respirator use did not alter the association between benzene exposure and hematologic outcomes. Therefore, neither of these variables was included in the subsequent analyses.

Forty-three of 44 benzene-exposed workers provided a spot urine sample at the end of a workshift (Factories 2, 3) or at the end of the high exposure period during their workshift (Factory 1). Samples were immediately aliquoted and frozen on dry ice, and transported back to the SHAC for storage and later shipment to an NCI biorepository in the United States.

The majority of subjects in the control factory that manufactured sewing machines were monitored for air levels of benzene, toluene, and xylene for 1 day on any of 6 different sampling days. No monitoring was performed in the second control workplace which was an administrative facility. Control subjects provided a spot urine sample at the SHAC during the clinical phase of the study.

Historical exposure assessment

Historical benzene exposure during subjects' employment at the study factories was estimated by trained field personnel using methods similar to those developed to assess historical exposure in the cohort study [Dosemeci et al., 1994]. This estimate incorporated work histories obtained by interview and review of workplace employment records, benzene area measurements obtained from factory records, and information on annual amount of benzene used, percent benzene used in workplace solvents, changes in manufacturing, and ventilation practices. Historical estimates considered current individual exposure levels when historical exposure monitoring data were sparse. Estimates of cumulative exposure to benzene were calculated from the historical time-specific exposure estimates and the duration worked. Exposure assessment was performed blinded with respect to hematologic measurements.

Interview and Biologic Sample Collection

Workers were evaluated at the SHAC. The study protocol was explained to all potential participants, and informed consent was obtained using Institutional Review Board-approved procedures. Eighty-eight of 94 (94%) eligible subjects selected for study agreed to participate. Each subject was administered a questionnaire by a trained interviewer from the staff of the Department of Occupational Health. Data collected included age, gender, current, and lifelong tobacco use, alcohol consumption, medical history, and an occupational work history. Height and weight of each subject were measured and a 27 mL sample of blood was obtained by venous phlebotomy. Peripheral blood films were made immediately after phlebotomy with blood that had not been anticoagulated. A spot urine sample was collected and analyzed by gas chromatography/mass spectrometry (GC/MS) for cotinine levels and adjusted for creatinine; these were highly correlated with self-reported current number of cigarettes used per day (Spearman $r = 0.86$, $p < 0.0001$).

Blood Cell Analysis

Blood samples were delivered within 4–6 hr after collection to the Huang Shan Hospital in Shanghai. Blood samples were analyzed by a Coulter T540 blood counter which was calibrated daily. Approximately 10^4 white blood cells (WBC; Coulter Corporation, personal communication) are counted to provide a total WBC count and a lymphocyte percentage, which are multiplied together to generate the absolute lymphocyte count. All abnormal counts were reviewed by hand. Five hematologic measurements are presented in this paper; the total WBC count ($\times 10^3/\mu\text{L}$ blood), the absolute lymphocyte count ($\times 10^3/\mu\text{L}$ blood), the hema-

tocrit (%), the red blood cell count (RBC; $\times 10^6/\mu\text{L}$ blood), and the platelet count ($\times 10^3/\mu\text{L}$ blood). The mean corpuscular volume (MCV; μm^3) was calculated as the hematocrit/(RBC count $\times 10$). The WBC differential was hand-counted on 100 cells by technicians at the American Medical Laboratories, Inc. (Chantilly, Virginia). Peripheral films were systematically reviewed by a hematologist (G.E.M.) and scored using a standardized detailed form for RBC, WBC, and platelet morphology. Since there were a substantial number of subjects with automated platelet counts below $100 \times 10^3/\mu\text{L}$ blood in both the exposed and control groups, the average number of platelets per high powered field was counted as a quality control check; there was a positive correlation (Spearman $r = 0.65$, $p = 0.001$) between these two measures.

Benzene Metabolite Measurements

Benzene urinary metabolites were measured (W.E.B.) using a modification of a previously described isotope dilution GC/MS assay [Bechtold et al., 1991]. ^{13}C -labeled analogues of the trans,trans-muconic acid (muconic acid), and phenol, catechol, and hydroquinone as sulfate and glucuronide conjugates, were used as internal standards. Concentrations were determined by calibrating against a primary standard solution. Urine samples were diluted with deionized water because of the high concentrations of metabolites. Thus, 200 μL of distilled water were added to vials with 200 μL of urine samples, followed by 100 μL of internal standard solution. The contents were mixed with 100 μL of concentrated HCl. The vials were capped and incubated at 100°C for 60 min, and allowed to cool. The solution was extracted with 2 mL of ethyl acetate. The ethyl acetate layer was removed and dried with a small scoop of sodium sulfate. The sample was decanted and the drying agent was rinsed with 1 mL of ethyl acetate. The ethyl acetate aliquots were combined and evaporated to 200 μL under nitrogen. The analytes were derivatized with 100 μL of bistrimethylsilyltrifluoroacetamide, incubated at 60°C for 30 min, and analyzed by GC/MS.

Statistical Methods

Arithmetic mean $\pm$ standard deviation (sd) and median with range are used to summarize data. Correlation analyses were performed with Spearman rank order correlation. The Wilcoxon rank sum test was used to test for differences in hematologic outcomes between exposed subjects and controls. Subsequent analyses were performed on three groups of study subjects: controls, subjects exposed to ≤ 31 ppm benzene (the median TWA exposure level), and subjects exposed to > 31 ppm benzene. The distribution of selected hematologic outcomes within these exposure categories is shown with Box and Whisker plots [Tukey, 1977]. Contin-

TABLE I. Demographic Characteristics of Workers Exposed to Benzene and Controls, Shanghai, China, 1992

	Exposed (n = 44) Mean $\pm$ sd	Controls (n = 44) Mean $\pm$ sd
Age (years)	35.3 $\pm$ 7.8	35.4 $\pm$ 7.3
Cigarettes/day		
Females (n=21)	0.0	0.0
Males (n=23)	11.0 $\pm$ 7.8	13.5 $\pm$ 13.7
Drinks of alcohol/week	1.5 $\pm$ 3.2	1.4 $\pm$ 2.2
Quetelet's index (weight _{kg} /height _{meters} ²)	21.6 $\pm$ 3.2	21.5 $\pm$ 2.3

uous outcome data that were not normally distributed were logarithmically transformed to improve normality. Analysis of covariance was used to test for group differences in continuous outcomes. The original matching variables, age and gender, were included in all models. Other variables tested for potential confounding or effect modification included current smoking status (average number of cigarettes per day in last month), current alcohol intake (categorized as 0 or ≥ 1 drink per week), and Quetelet's index, a measure of obesity calculated as weight_{kg}/height_{meters}².

Unconditional logistic regression was used to test for differences in the proportion of subjects with a peripheral blood cell morphologic abnormality among exposed workers vs. controls, and to evaluate potential confounders. Two-sided *p* values were calculated throughout: *p* values < 0.05 were considered adequate to reject the null hypothesis. All statistical analyses were performed using SAS for Personal Computer version 6.04 [SAS Institute Inc., 1987].

RESULTS

Subject Characteristics

Forty-four workers currently exposed to benzene and 44 never-exposed controls were enrolled in the study. Age, alcohol intake, Quetelet's index, and daily cigarette use were comparable between exposed and control subjects (Table I). Twenty-one of 44 (48%) subjects in each group were female.

Exposure Assessment

Mean (sd) years of occupational exposure to benzene among the exposed subjects were 6.3 (4.4), with a range of 0.7–16 years. Benzene comprised 30–44% of the primary solvent mixture used in the exposed factories. The median 8-hr TWA benzene personal air level was 31 ppm. Exposure to toluene and xylene was low, relative to benzene (Table II). As shown in Table III, the amount of each benzene

metabolite in urine was substantially higher among exposed than control workers. Among the 38 exposed subjects monitored for both air exposure and urine metabolite levels on the same day, there was a significant correlation (*p* < 0.0001) between benzene air levels and phenol, muconic acid, hydroquinone, and catechol urine levels (*r* = 0.71, 0.66, 0.68, and 0.66, respectively). Similar correlations were observed in men and women (e.g., for phenol: *r* = 0.69, *p* = 0.002 among 17 men and *r* = 0.61, *p* = 0.003 among 21 women).

Also noteworthy, the relative increase in metabolite level in the group exposed at greater than the median benzene exposure value (31 ppm) compared to those exposed to less than this amount was about 9-fold for phenol and catechol and only 5 to 6-fold for hydroquinone and muconic acid (Table III). The proportion of all measured benzene metabolites excreted as muconic acid and hydroquinone decreased from a median (range) of 32% (21–42%) in workers exposed to ≤ 31 ppm benzene to 24% (14–39%) in workers exposed to >31 ppm (*p* = 0.006 by Wilcoxon rank sum test).

Hematologic Measurements

Automated peripheral blood cell counts

The absolute lymphocyte count was the most significantly decreased blood cell measure in the exposed group compared to controls (mean [sd] 1.5 [0.3] $\times 10^3/\mu\text{L}$ vs. 1.9 [0.4] $\times 10^3/\mu\text{L}$, *p* < 0.0001). Other measures also decreased were the platelet count (127 [44] $\times 10^3/\mu\text{L}$ vs. 166 [59] $\times 10^3/\mu\text{L}$, *p* = 0.001), WBC count (6.0 [1.9] $\times 10^3/\mu\text{L}$ vs. 6.8 [1.7] $\times 10^3/\mu\text{L}$, *p* = 0.006), RBC count (4.4 [0.6] $\times 10^6/\mu\text{L}$ vs. 4.7 [0.6] $\times 10^6/\mu\text{L}$, *p* = 0.01), and hematocrit (40.0% [5.6] vs. 42.0% [5.6], *p* = 0.10), while the MCV was significantly elevated (91.4 [4.0] μm^3 in exposed subjects compared to 88.9 [4.9] μm^3 in controls, *p* = 0.02). These differences were minimally changed after adjustment for alcohol consumption, cigarette use, and Quetelet's index.

Comparable results were observed in men and women. For example, the absolute lymphocyte count was a more sensitive measure of benzene exposure than the total WBC count in both groups. The total WBC count ($\times 10^3/\mu\text{L}$) was 9% lower in exposed women vs. female controls (mean [sd] 6.1 [2.0] vs. 6.7 [2.2], *p* = 0.16), while the absolute lymphocyte count ($\times 10^3/\mu\text{L}$) was 22% lower in exposed women compared to female controls (1.4 [0.4] vs. 1.8 [0.3], *p* = 0.002). Similarly, the total WBC count was 12% lower in exposed men vs. male controls (6.0 [1.8] vs. 6.8 [1.2], *p* = 0.01), while the absolute lymphocyte count was 25% lower in exposed men compared to male controls (1.5 [0.32] vs. 2.0 [0.4], *p* = 0.0005).

Table IV portrays summary values for blood counts in

TABLE II. Current Benzene, Toluene, and Xylene 8-Hr TWA Personal Air Levels (ppm), by Exposure Category, in a Study of Workers Exposed to Benzene in Shanghai, China, 1992

	Benzene (ppm) Median (range)	Toluene (ppm) Median (range)	Xylene (ppm) Median (range)
Control (n=44) ^a	0.02 (0.01–0.1)	0.02 (0.02–0.3)	0.02 (0.02–0.6)
Exposed			
≤ 31 ppm (n = 22) ^b	13.6 (1.6–30.6)	0.02 (0.01–9.7)	0.02 (0.02–12.8)
> 31 ppm (n = 22)	91.9 (31.5–328.5)	0.1 (0.02–12.9)	0.2 (0.03–7.2)

^aAir samples obtained for 31 of 44 controls (one sample).^bAir samples for exposed subjects represent geometric mean of five samples.**TABLE III.** Benzene Urine Metabolites, by Exposure Category, in a Study of Workers Exposed to Benzene in Shanghai, China, 1992*

	Phenol Median (range)	Catechol Median (range)	Muconic acid Median (range)	Hydroquinone Median (range)
Control (n=44) ^a	17.3 (3.7–55.3)	3.2 (1.6–8.7)	0.18 (0.1–0.8)	1.6 (0.9–6.7)
Exposed				
≤31 ppm (n = 22) ^b	38.9 (15.2–217.1)	7.0 (2.5–38.9)	8.15 (2.3–55.5)	12.8 (3.2–61)
>31 ppm (n = 22) ^c	349.7 (36.8–516.7)	65.7 (5.0–84.2)	46.8 (8.6–103.4)	64.3 (9.7–196.6)

*Units µg/mg creatinine for all metabolites.

^aA subsample of 17 controls frequency-matched on age and gender to the exposed workers were tested for urine metabolites in urine collected while at the SHAC clinic.^bTwenty-two/22 subjects provided a urine sample.^cTwenty-one/22 subjects provided a urine sample.

unexposed controls and among subjects exposed to less than and greater than the median benzene level (31 ppm). The respective absolute lymphocyte count and MCV distributions are portrayed in Figures 1 and 2. All blood parameters were significantly different between controls and subjects exposed to >31 ppm (Table IV). The magnitude of the difference was largest for the absolute lymphocyte count (32% decrease) and smallest for the MCV (4% increase). Only the absolute lymphocyte count, RBC count, and platelet count were significantly different between controls and subjects exposed to ≤31 ppm benzene. These group differences were minimally changed after adjustment for alcohol consumption, cigarette use, and Quetelet's index.

Since the lower exposed group (≤31 ppm) contained some subjects with 8-hr TWA exposures above 31 ppm on at least one of five sampling days, a subgroup was created that contained subjects who never had a sample above 31 ppm on any sampling day (n = 11, median 8-hr TWA = 7.6 ppm, range = 1–20 ppm). Only the absolute lymphocyte count differed significantly between this subgroup and controls (mean [sd] 1.6 [0.4] vs. 1.9 [0.4], p = 0.03).

In an analysis restricted to the benzene-exposed workers, we examined the strength of the exposure-response re-

lationship over the entire range of exposures as indicated by individual air and urinary metabolite measurements (Table V). The urine metabolite data complement exposure assessed by the 8-hr TWA benzene air measurements since they incorporate interindividual variation in respirator use, percutaneous absorption of benzene, and metabolism. Only the total WBC count, absolute lymphocyte count, and MCV showed evidence for a dose-response relationship with these measures of benzene exposure. Similar results were generally found using the 8-hr TWA and urine metabolite data (Table V). Although the correlation between each benzene metabolite and the absolute lymphocyte count was similar (Table V), only the association with hydroquinone achieved statistical significance. In contrast, catechol was more highly correlated with the MCV (r = .53, p = 0.0001) than the other three benzene metabolites (range: r = 0.30 to r = 0.36) (Table V) for the entire group. Various combinations of urine metabolites had no stronger association with the hematologic outcomes than did levels of individual metabolites. Neither estimated cumulative life-time benzene exposure nor number of years worked in an exposed factory was significantly associated with any hematologic outcome (data not shown).

TABLE IV. Comparison of Peripheral Blood Counts, By Exposure Status, in a Study of Workers Exposed to Benzene in Shanghai, China, 1992*

	WBC	Absolute lymphocyte count	RBC	Hematocrit	MCV	Platelets
	Mean $\pm$ sd (range)	Mean $\pm$ sd (range)	Mean $\pm$ sd (range)	Mean $\pm$ sd (range)	Mean $\pm$ sd (range)	Mean $\pm$ sd (range)
Control (n = 44)	6.8 $\pm$ 1.7 (3.1–12.5)	1.9 $\pm$ 0.4 (1.1–2.8)	4.7 $\pm$ 0.6 (3.0–5.6)	42.0 $\pm$ 5.6 (27.7–50.7)	88.9 $\pm$ 4.9 (71.2–96.6)	166 $\pm$ 59 (62–313)
Exposed						
≤31 ppm (n = 22)	6.4 $\pm$ 1.8 (3.5–11.1)	1.6 $\pm$ 0.3 (1.1–2.5) ^a	4.6 $\pm$ 0.6 (3.2–5.7) ^b	41.2 $\pm$ 5.7 (29.5–49.7)	89.8 $\pm$ 3.9 (80.1–99.8)	132 $\pm$ 45 (59–204) ^b
>31 ppm (n = 22)	5.6 $\pm$ 1.9 (3.8–11.4) ^a	1.3 $\pm$ 0.3 (0.9–2.3) ^c	4.2 $\pm$ 0.6 (3.5–5.3) ^c	38.8 $\pm$ 5.3 (31.6–50.9) ^b	92.9 $\pm$ 3.4 (85.7–98.3) ^c	121 $\pm$ 43 (65–216) ^a

*Units: WBC count ($\times 10^3/\mu\text{L}$ blood), absolute lymphocyte count ($\times 10^3/\mu\text{L}$ blood), RBC count ($\times 10^6/\mu\text{L}$ blood), hematocrit (%), platelet count ($\times 10^3/\mu\text{L}$ blood), MCV (μm^3).

^a $p < 0.01$.

^b $p < 0.05$.

^c $p < 0.001$.

Peripheral blood film differential and morphology

The proportion of granulocytes in the WBC differential count increased from 64% to 71% between controls and subjects in the higher exposure group, lymphocytes decreased from 27% to 23%, and basophils decreased from 0.5% to 0.1%. The percent monocytes and eosinophils were similar between these two groups, and no left shift among the granulocytes was observed (data not shown). A direct comparison of granulocyte vs. lymphocyte toxicity was made using data from the manual differentials by comparing the percent granulocytes times the total WBC count with the percent lymphocytes times the total WBC count. The absolute granulocyte count ($\times 10^3/\mu\text{L}$) decreased only 7%, from mean (sd) 4.3 (1.7) among workers in the ≤ 31 ppm group to 4.0 (1.8) among workers in the higher exposure group ($p = 0.28$), whereas the absolute lymphocyte count ($\times 10^3/\mu\text{L}$) decreased 25%, from 1.6 (0.6) to 1.2 (0.6) ($p = 0.02$).

There were no significant differences observed overall or for specific types of morphologic abnormalities in lymphocytes, granulocytes, or platelets between controls and either of the two exposure groups (Table VI). In contrast, there was a significant difference in the proportion of subjects among the higher exposed group with any RBC abnormality compared to controls (86% vs. 50%, $p < 0.01$). Significant elevations were seen specifically for stomatocytes and target cells in the more highly exposed workers (Table VI). These differences were minimally changed after adjustment for alcohol consumption, cigarette use, and Quetelet's index.

DISCUSSION

Forty-four workers exposed to a wide range of benzene were found to have decreased levels of all blood elements tested, as compared to controls. The absolute lymphocyte count was the hematologic measure most significantly different between exposed and control subjects, and was the only blood cell type significantly lower in a subgroup of workers with median TWA = 7.6 ppm (range 1–20 ppm) compared to controls. Other hematologic parameters were altered in the exposed group compared to controls and are consistent with hematotoxic effects of benzene reviewed by Aksoy [1988] and Goldstein [1977], i.e., toxicity for WBC, platelets, RBC, and a tendency towards macrocytosis. However, in our study, none of these measures was as sensitive an indicator of benzene exposure as the absolute lymphocyte count, particularly at levels ≤ 31 ppm.

Among the benzene-exposed workers, only the WBC count, absolute lymphocyte count, and MCV were significantly correlated with continuous (vs. categorized) measures of benzene exposure. The finding of overall decreases among benzene-exposed workers of other peripheral blood cells, and yet the lack of a dose-response relationship, may reflect a more idiosyncratic reaction to benzene exposure. In addition, in morphologic studies, we found that only RBC abnormalities, and not those of any type of WBC or platelet, were associated with benzene exposure, and only among the most heavily exposed workers.

Goldwater [1941] noted that lymphocytopenia was more frequently found among subjects exposed to benzene than was leukopenia. Aksoy found that 28 of 37 leukopenic

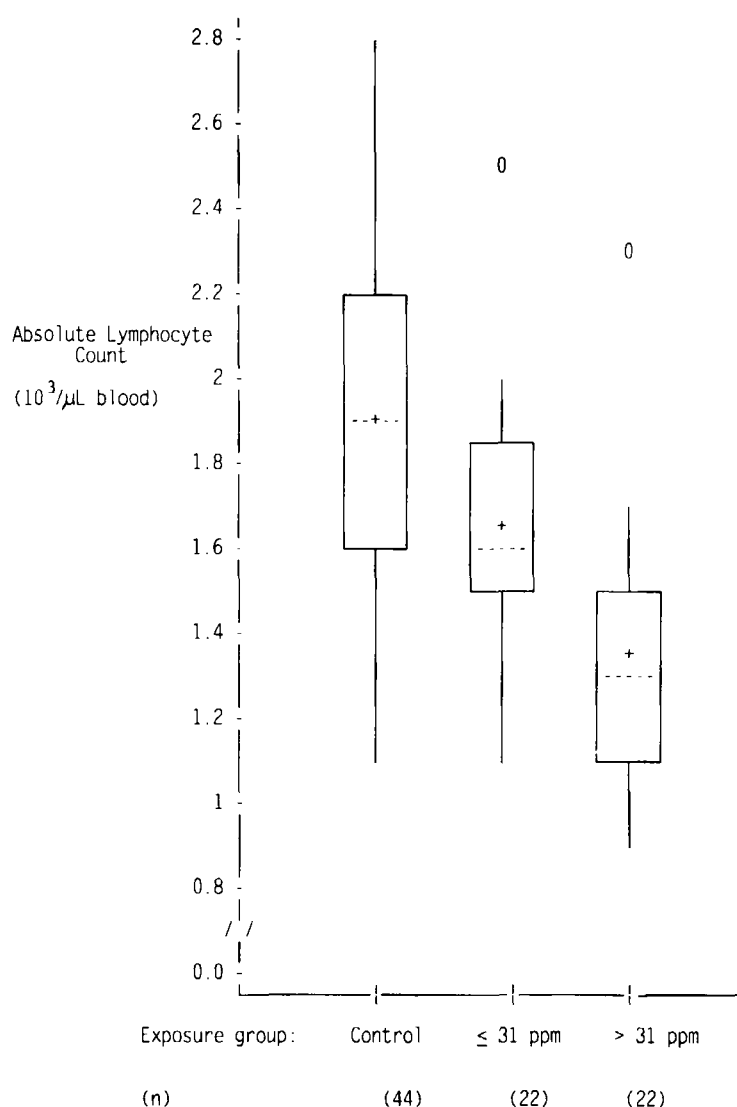


FIGURE 1. Absolute lymphocyte count, by current benzene exposure category, in a study of workers exposed to benzene in Shanghai, China, 1992. Upper edge of box represents highest 25th percentile of data. Lower edge of box represents 75th percentile. Upper and lower lines

(whiskers) indicate range of remaining data except for outliers. O, outlier defined as >1.5 and $<3.0 \times$ distance between 25th and 75th percentiles. +, mean; -----, median.

patients exposed to benzene also had lymphocytopenia [as described in Aksoy, 1988], though he did not address the relative sensitivities of these two measures for benzene toxicity. Yin et al. [1987] reported that the absolute lymphocyte count and leukocyte count were similarly reduced in workers exposed only to benzene. In contrast, several authors have reported either no association between benzene and the absolute lymphocyte count, or lymphocytosis [Bernard, 1942; Hamilton-Patterson and Browning, 1944; Hernberg et al., 1966; Doskin, 1971; Aksoy et al., 1971].

Although our study is relatively small compared to some of these previous reports, its strengths include comparison to matched controls, strict blinding in the analysis,

detailed recent and historical exposure data, a population exposed to benzene with relatively low levels of toluene exposure, and absolute lymphocyte counts measured by an automated Coulter counter. Further, we demonstrated a dose-response relationship between the absolute lymphocyte count and benzene air level and benzene urine metabolites, and evaluated each association for potential confounding. Our findings are consistent with results of animal studies demonstrating sensitivity of lymphatic tissue to benzene [Snyder et al., 1980; Ward et al., 1985] at concentrations as low as 10 ppm benzene [Baarson et al., 1984], with numerous reports that have described an excess of chromosome damage in lymphocytes from workers exposed to ben-

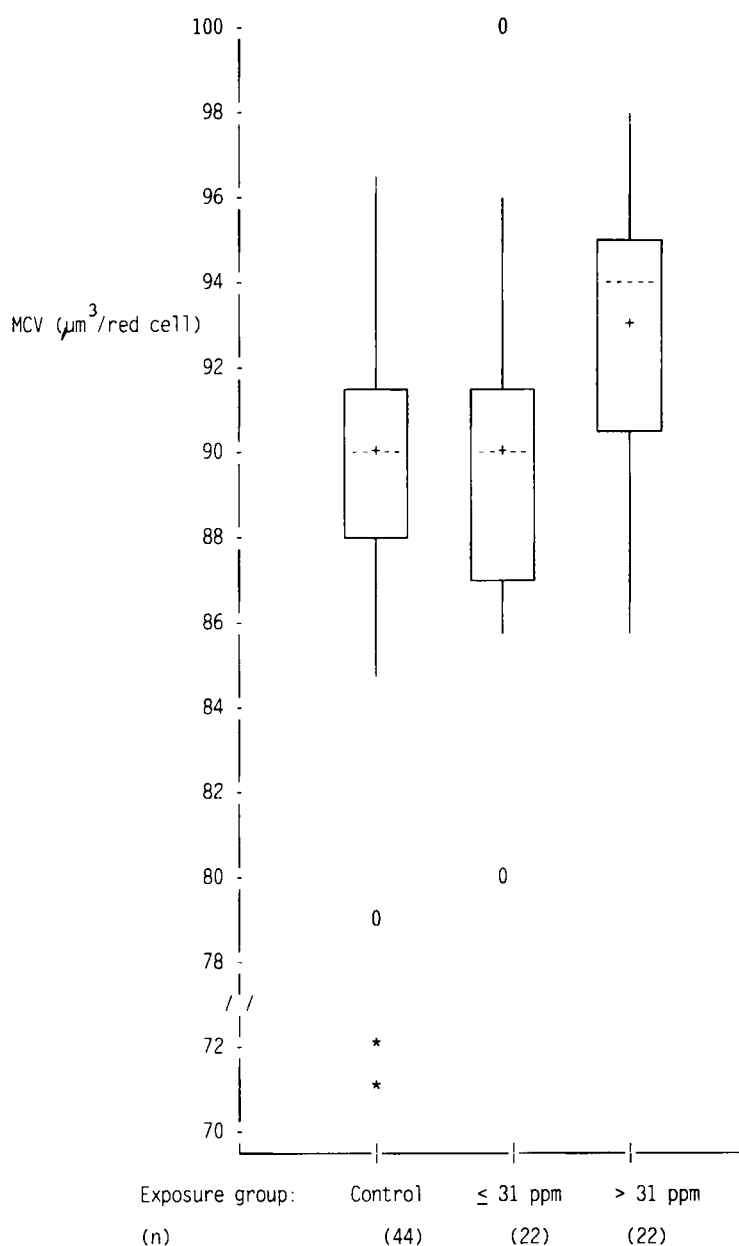


FIGURE 2. Red blood cell mean corpuscular volume, by current benzene exposure category, in a study of workers exposed to benzene in Shanghai, China, 1992. Symbols as for Fig. 1., plus *extreme outlier defined as $>3.0 \times$ distance between 25th and 75th percentiles.

zene [e.g., Forni et al., 1971a,b; Picciano, 1978; Ding et al., 1983; Sarto et al., 1984], and with findings from several studies which suggest that benzene is associated with excess risk of hematopoietic tumors derived from lymphocytes [Rinsky et al., 1987; Wong, 1987; La Vecchia et al., 1989; Blair et al., 1993; Yin et al., 1995].

Most recent hematologic studies of workers exposed to benzene and unexposed controls have not reported data on the absolute lymphocyte count, including studies of workers exposed up to 1.4 ppm [Collins et al., 1991], 10 ppm [Yardley-Jones et al., 1988], 12.4 ppm [Sarto et al., 1984], 31.5

ppm [Hancock et al., 1984], and 35 ppm benzene [Townsend et al., 1978]. These authors generally concluded that there is little evidence of benzene-induced hematotoxicity at these exposure levels, with the possible exception of mild macrocytosis [Collins et al., 1991; Yardley-Jones et al., 1988] or small declines in the RBC count [Townsend et al., 1978]. Given the limited published data on the absolute lymphocyte count in workers exposed to benzene in the 1–30 ppm range, particularly in the presence of other aromatic compounds such as toluene, it is difficult to evaluate the sensitivity of this measure at these exposure levels.

TABLE V. Correlation of Peripheral Blood Counts With Various Measures of Benzene Exposure Among Exposed Workers, Shanghai, China, 1992*

Measures of benzene exposure	WBC (R)	Absolute lymphocyte count (R)	RBC (R)	Hematocrit (R)	MCV (R)	Platelets (R)
Air measurement						
8-Hr TWA	-0.35 ^a	-0.38 ^a	-0.06	-0.01	0.33 ^a	-0.13
Urinary metabolites						
Phenol (n=43)	-0.23	-0.31	-0.09	0.00	0.30	-0.02
Muconic (n=43)	-0.23	-0.30	-0.11	0.00	0.32 ^a	-0.0006
Hydroquinone (n = 43)	-0.26	-0.36 ^a	-0.084	0.00	0.36 ^a	0.016
Catechol (n = 42)	-0.28	-0.25	-0.14	0.00	0.53 ^b	-0.048

*Correlation calculated by Spearman rank order correlation for all outcomes except hematocrit and RBC count; for these, correlation coefficients adjusted for age and sex were determined from a linear regression model.

^ap < 0.05.

^bp < 0.001.

TABLE VI. Peripheral Blood Cell Morphology, by Exposure Category, in a Study of Workers Exposed to Benzene in Shanghai, China, 1992

	Granulocyte abnormality No. abnormal/ total	Lymphocyte abnormality No. abnormal/ total	Platelet abnormality No. abnormal/ total	RBC abnormality No. abnormal/ total	Stomatocytes No. abnormal/ total	Target cells No. abnormal/ total	Anisocytosis No. abnormal/ total
Control (n = 42)	3/42 (7%)	7/40 (17%)	9/40 (22%)	21/42 (50%)	11/42 (26%)	6/42 (14%)	22/42 (52%)
Exposed							
≤31 ppm (n = 22)	1/20 (5%)	2/21 (10%)	5/19 (26%)	8/22 (36%)	6/22 (27%)	4/22 (18%)	15/22 (68%)
>31 ppm (n = 22)	0/21 (0%)	1/21 (5%)	5/21 (24%)	18/21 (86%) ^a	12/21 (57%) ^b	8/21 (38%) ^b	16/21 (76%)

^ap < 0.01.

^bp < 0.05.

In our study, the proportion of measured benzene metabolites excreted as muconic acid and hydroquinone decreased from 32% in workers exposed to ≤31 ppm benzene to 24% in workers exposed to >31 ppm. The tendency to form less muconic acid and hydroquinone relative to phenol and catechol at higher benzene levels has also been observed in several animal studies [Henderson et al., 1989; Sabourin et al., 1989]. The risk-assessment implications of shifts in benzene metabolite formation are uncertain, however, since the relative contribution that each metabolite makes to the genotoxic effects of benzene is still a subject of some debate. While several studies have shown that benzene metabolites related to muconic acid (e.g., trans, trans-muconaldehyde) and hydroquinone and its related compounds (e.g., *p*-benzoquinone) are more genotoxic than phenol and catechol in some tests systems [Irons and Nep-tun, 1980; Rushmore et al., 1984; Schwartz et al., 1985;

Eastmond et al., 1987; Snyder et al., 1989; Glatt et al., 1989], catechol has been shown to be a more potent inducer of sister chromatid exchange than hydroquinone [Erexson et al., 1985; Glatt et al., 1989; Morimoto et al., 1993]. Further, phenol [Subrahmanyam et al., 1990] has been shown to stimulate hydroquinone bioactivation and potentiate hydroquinone-induced myelotoxicity [Eastmond et al., 1987], and catechol has been shown to stimulate hydroquinone bioactivation [Subrahmanyam et al., 1991] and potentiate hydroquinone-induced micronuclei formation [Robertson et al., 1991]. Thus, the complex nature of benzene metabolite interactions precludes firm conclusions about the relevance of alterations in benzene metabolite formation at different levels of benzene exposure.

Since the four benzene urine metabolites measured in this study were highly correlated (at about 90%), we had limited ability to evaluate the relative impact of each me-

tabolite on hematologic outcomes. Catechol was, however, more strongly associated with the MCV, a measure of RBC toxicity, and hydroquinone was slightly more strongly correlated with the absolute lymphocyte count. No combination of metabolites was more strongly associated with peripheral blood cells than individual metabolites.

Individual benzene air levels in these factories were much higher than we had expected to find, based on historical area monitoring data. As a direct result of our study, remedial action was taken at the two workplaces with the highest benzene exposures, including substitution of toluene for benzene, enclosure of reaction vessels, and improvement in ventilation. Exposure patterns in the three factories evaluated in this study are not representative of general exposure patterns in China today. Based on data from 680 factories in 12 cities in China [Dosemeci et al., 1994], average exposure was 33 ppm from 1965 to 1969, decreasing to 8 ppm by 1985–1987. Recent exposure data collected from workplaces in Shanghai indicate that benzene levels have continued to decline in many factories up to the present time (Wang, Y.Z., unpublished data).

Current exposure to benzene in the United States is regulated at 1 ppm as an 8-hr TWA with a 15-min short-term exposure limit of 5 ppm [OSHA, 1987]. Although this level of benzene exposure is not considered hematotoxic, acute high-dose exposures are still being reported in the United States [e.g., Midzenski et al., 1992]. Our results support Goldstein's recommendation that close attention should be paid to the absolute lymphocyte count when monitoring workers for early biologic effects due to benzene exposure [Goldstein, 1988]. Although small changes in the absolute lymphocyte count are unlikely to have any short-term clinical consequences per se, this readily measurable and inexpensive marker may identify occupational environments that place workers at particularly increased risk for subsequent development of hematopoietic malignancies.

LaMontagne and colleagues [1993] have reviewed the use of the complete blood count with differential as a screening tool. They note that automated differential cell counters are likely to eliminate many of the sources of error present in manually performed WBC differentials, which are generally performed on only 100 WBCs (also commented on by Goldstein, 1988). They recommend that at least 400 WBCs be evaluated when manual counts are necessary. In our study, the automated absolute lymphocyte count ($\times 10^3 \mu\text{L}$) was significantly lower among workers in the ≤ 31 ppm exposure group compared to controls (mean [sd] 1.6 [0.3] vs. 1.9 [0.4], $p = 0.006$, 16% decrease), while the absolute lymphocyte count derived from a standard manual count of 100 cells was similar among exposed and control subjects (1.6 [0.6] vs. 1.7 [0.5], $p = 0.55$).

Finally, recent studies suggest that benzene may alter patterns of particular lymphocyte subsets [Zeman et al., 1990; Luan, 1992]. It is warranted to further explore these

findings at current levels of benzene exposure, particularly since very accurate lymphocyte subset analysis by flow cytometry has become widely available in the United States.

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