APPENDICES AVAILABLE ON REQUEST

Research Report 115

Validation and Evaluation of Biomarkers in Workers Exposed to Benzene in China

Appendix A. Analyses of the Combined Data for Year 1 and Year 2

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NYU-HEI BENZENE STUDY: REPORT OF THE ANALYSES OF THE COMBINED YEAR-1 AND YEAR-2 DATA

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Statistical Methods

Essentially the same statistical methods were used for the combined year-1 and year-2 data as had been used for the year-1 data. All analyses were conducted using SPSS (1994) or SAS (2000) statistical software. When appropriate, the continuous outcome variables were analyzed using a logarithmic or square-root transformation. For the chromosome aberration counts the square-root transform $(x + 3/8)^{1/2}$ was applied. This transformation is recommended for count data in order to achieve approximate normality and variance stabilization (Zar, 1984). For the several chromosome-aberration variables for which there were very few nonzero observations no analyses are presented. The urinary benzene metabolite variables (S-PMA, t,t-MA and phenol) were measured as continuous variables but proved to be right-skewed. A logarithmic transformation ($\ln[x+1]$) was therefore applied to these variables for analysis. Similarly, a logarithmic transformation was applied to a few skewed differential blood counts (monocytes, eosinophils and basophils) and to albumin adducts.

One-way analysis of variance, including a contrast to evaluate trends, was applied to compare multiple ordered exposure groups. The trend contrast scores consisted of successive values incremented by unity and centered around zero (e.g., -2, -1, 0, 1, 2). As such, it was a nonparametric trend test but did not take into account the relative differences between the mean exposure levels. Multiple regression was employed to analyze benzene exposure as a continuous variable which modeled the actual measured exposures.

The benzene exposure variable used for the primary analyses of chromosome aberrations and blood parameters was the mean of several personal measurements over a 4-week period (called "4-week" benzene exposure hereafter), including the measurement on the day the blood and urine samples were drawn plus measurements in the preceding 3-4 weeks. Of the 130 benzene-exposed workers, 117 (90%) had 4 measurements, 11 (8.5%) had 3 measurements and 2 (1.5%) had 2 measurements. The blood-count and chromosome-aberration data were also analyzed in relation to estimated cumulative occupational exposure to benzene (ppm-years). The cumulative occupational exposure information was based on industrial hygiene and other work records.

For the urinary metabolite measures, the primary benzene exposure variable was the personal measurement on the day of the urine samples, while the main outcome variables were the urine metabolite levels at the end of the workday and the difference scores (the after-work measurement minus the before-work measurement).

The benzene exposure data contained one individual with exposure levels more than twice as high as anyone else. Because this observation had the potential to be highly influential in regression analyses, this influence was lessened by arbitrarily reassigning his exposure values to be 20% greater than the next highest value. In addition, because the exposure data were right skewed, a semi-parametric analysis based on exposure ranks and outcome-variable ranks was conducted to see if it corroborated the parametric analyses.

The multiple regression analyses evaluated several covariates as potential confounders, including sex, age, cotinine levels and toluene exposure. These were routinely incorporated into the analyses. Because of the right skewness of the cotinine and toluene values, logarithmic transformations of these variables were used in the analyses. In addition, sub-analyses were performed to evaluate the impact of the potential confounding variables on the results, especially in the case of toluene, which at high doses can compete with benzene for binding sites.

Recent Changes in the Analytic Data Set

In previous reports of this study the differential blood counts had been based on only 100 cells. In order to have more assurance in the findings regarding these endpoints, we had an additional 800 cells counted, so the present results are based on counting 900 cells per subject.

The levels of benzene and toluene among nominally "unexposed" subjects were all below the Lower Limit of Detection (LLD). We had previously coded these all as zero. We have now determined that the LLD for the personal samplers was about 0.01 ppm for both benzene and toluene. Therefore, for the present analyses, for each unexposed subject random values (assuming a uniform distribution over the range 0 to 0.01) were generated for benzene and toluene. These values were used for all exposure-response analyses, other than the cumulative exposure analyses for which zero was used.

We suspected there was under-reporting of smoking. When we compared the cotinine distributions for reported smokers and nonsmokers, there were several outliers among the reported nonsmokers that produced appreciable overlap with the reported smokers. Based on the lower limits of the distribution among reported smokers and the "outliers" among the "nonsmokers," we decided to assign all reported nonsmokers with >100 mcg cotinine as smokers. No reported smokers were reclassified.

Comparison of Year-1 and Year-2 Data

Before combining the data collected in the first and second years of the study, it is

important to evaluate the comparability of the biomarker measurements over those two years. Since by design the exposure levels were not comparable for the two years, the comparisons are limited to the unexposed control groups in the first set of analyses. Table 1 shows the results of this comparison. The year-1 and year-2 groups were comparable with regard to demographics and lifestyle. They did not differ in age, sex or current smoking. Although there were several statistically significant differences between the two groups with regard to the urine biomarkers (t,t-MA, S-PMA and phenol) these differences were small compared to the range of values in the exposed groups, so are probably not a source of concern. There were few differences between the two years with regard to blood counts or chromosome aberrations. All in all, the differences in the biomarker measures were few and mostly small.

To further evaluate the comparability of the year-1 and year-2 data, outcome variables for subjects who had exposure levels in a comparable range for years 1 and 2 (6-20 ppm for 4-week average benzene exposure for the blood-based variables, or 6-30 ppm for current-day exposure for the urine metabolite variables) were compared. Years 1 and 2 were compared using analysis of covariance to control for exposure levels. These analyses included 9 year-1 and 23 year-2 subjects for the blood variables, and 14 and 15 respectively for the urine variables. For the 31 outcome variables compared (7 blood counts, 2 albumin adduct counts, 13 indications of chromosome aberrations, and 9 urine metabolite variables), 7 showed a statistically significant difference. However, the differences were not consistent (year-1 values were higher than year-2 values for chromatid gaps, hypodiploidy (45 chromosomes), aneuploidy (45 or 47 chromosomes) and t,t-MA after work, but lower than year-2 values for eosinophils, enhanced hypodiploidy (<45 chromosomes) and S-PMA after work). In addition, some of the variables were intrinsically correlated with each other, so there were fewer than 7 independent significant results.

Since there were no clear indications of consistent differences between the year-1 and year-2 subjects, there seemed to be justification to combine the two datasets for analysis. In general, it was not thought appropriate to control for year in the analysis of the data because the exposure levels were much higher in year 1 (mean = 29.7 ppm) than in year 2 (mean = 5.2 ppm), so if year is included in the analysis it can potentially severely confound the exposure level and give distorted results with respect to exposure effects. However, a series of methodological analyses (see Table 21) showed that its effects were relatively small, so it was not viewed as a serious concern and was not included in most analyses.

Benzene and Other Exposure Levels

Frequency distributions for several of the exposure variables are shown in Table 2. Our intention was to have a substantial number of subjects with benzene levels between 0 and 1 ppm. However, when the benzene exposure measurements were completed, it turned out that many were exposed to 1-3 ppm instead. The means, standard deviations and medians for several exposure variables are shown at the bottom of Table 2. The exposure distributions tended to be skewed, as shown by the large standard deviations (SDs) and the discrepancies between the mean

and the median. In addition to the measures shown in Table 2, the distribution of "lifetime" cumulative occupational benzene exposure is shown in Table 3. The mean and SD for cumulative benzene exposure proved to be 81.3 ± 89.3 ppm-years, with a median of 51.2. Toluene levels were quite high, with a median of 12.6 ppm and a mean of 26.3 ppm (Table 2). The levels of xylene were low (mean and SD of 0.40 ± 0.41 and median of 0.30).

Because people are sometimes not accurate in reporting their smoking habits, we examined the distribution of urine cotinine levels to determine if there were false negatives in the reporting of smoking. Based on the distributions in the nonsmoker and smoker groups, it was decided to recode all the reported nonsmokers with cotinine levels (mean of two urine samples) >100 μg/g creatinine as smokers.

Other Analyses

It can be observed in Table 4 that the unexposed and exposed groups were very similar in their sex distribution. Once cotinine was used to reclassify smoking status for a few people, the comparability in smoking was fairly good. The exposed group on average was slightly older than the unexposed group.

Table 4 shows the means in the unexposed group and the total exposed group for the variables analyzed in the study. (Several additional blood-differential and chromosome variables – metamyelocytes, microcytosis, macrocytosis, poikilocytosis, hypochromia, polychromasia, chromosome gaps, minute deletions, dicentrics and rings -- were excluded because they occurred too rarely). Clear differences between the exposed and unexposed groups are seen for all of the urine metabolites and albumin adduct measures, for several of the blood counts, and for a few of the chromosome aberration and aneuploidy variables.

Results for Benzene Urinary Metabolite Analyses

Tabulations of Urinary Metabolite Levels. All of the urine metabolite variables were corrected for urinary creatinine levels. Table 4 compares the exposed and unexposed groups with regard to selected urine biomarker variables. It is obvious that overall there were large differences associated with benzene exposure. Table 5 shows the urine biomarker data broken down into graded exposure categories, where the pertinent benzene exposure was taken to be the exposure measurement for the current day. For t,t-MA, and especially S-PMA, there are increases in these metabolites even at exposure levels under 1 ppm. Phenol, on the other hand, shows an increase only for benzene exposure levels >5 ppm. In virtually all cases there was a highly statistically significant positive exposure-response trend over the whole range of exposures (although the exposure-response trends shown in this table were not adjusted for any possible confounding variables).

Exposure-Response Analyses. Table 6 summarizes multiple regression analyses of the urinary metabolite biomarkers on benzene exposure. These analyses assessed exposure-response

relationships with adjustment for confounders. The possible confounding variables that were considered were sex, age, cotinine levels and toluene exposure levels. There was particular concern that toluene might serve to confound the benzene effect. However, a comparison of the results with and without toluene in the analysis in Table 6 shows that toluene's impact was small and that the predominant effect was that due to benzene exposure. (A further discussion of toluene as a potential effect modifier, i.e., an interacting variable, is given below in relation to Tables 19 and 20.) There was no evidence that smoking, gender or age were confounders for the urinary metabolite variables (Table 6).

Table 7 shows the results for low exposure levels (<1 ppm). It is notable that both t,t-MA and S-PMA show highly significant exposure-response trends even over the exposure range of 0 to 1 ppm. Furthermore, an examination of the exposed group with ≤0.5 ppm showed a statistically significant increase as compared to the unexposed group for S-PMA at the end of the workday.

Shape of Exposure-Response Curve. Table 8 provides information on whether the associations between benzene exposure and various urine biomarkers are linear or curvilinear. This was done by fitting models that included both linear and dose-squared terms for benzene exposure. At the outset it should be noted that the scale of the biomarker variable may play a consequential role in the shape of the exposure-response curve such that an alternate transformation of the biomarker variable could change the shape of the curve. We therefore tested the direction and significance of the dose-squared component of the linear-quadratic curves for the urine biomarkers using three metrics: arithmetic exposure and logarithmic biomarker, logarithmic exposure and logarithmic biomarker, and arithmetic exposure and arithmetic biomarker.

As it turns out, most of the t,t-MA and S-PMA biomarkers in Table 8 which show negative dose-squared terms (which implies convex curvilinearity, i.e., a proportionally greater difference at low doses than at high doses), show it with all three metrics used. Thus, the low-exposure sensitivity of t,t-MA and S-PMA as biomarkers of benzene exposure (and/or the partial saturation of biomarker effects at high benzene doses) is shown by the convex curvature of their responses.

For phenol, the post-work and post-pre work measures were less sharply curvilinear. Thus phenol has less of the convex curvature that indicates low-exposure sensitivity than the other urine biomarkers have. This same feature is shown in the exposure-response table (Table 5), in which there is not a clear increase in phenol levels for exposures below 5 ppm.

Results for Blood Cell Counts

The pertinent benzene exposures for blood counts, albumin adducts and chromosome aberrations and aneuploidy are considered to be the average exposure level over four weeks, since the effects upon the hematopoietic system are much longer lived than are urinary metabolites. Additional analyses related the blood and chromosome outcomes to cumulative lifetime

occupational benzene exposure, which may be important for long-lived blood cells and for stable chromosome aberrations.

Tabulation of Blood Counts by Benzene Exposure Levels. Table 9 tabulates the overall exposure-response associations for average 4-week benzene exposure and various blood counts. There were significant exposure-related decreases in red blood cells, white blood cells and neutrophils. Somewhat surprisingly, there was no clear benzene effect upon lymphocytes in this analysis, nor was there any effect upon platelets, monocytes, eosinophils, basophils, bands or atypical lymphocytes. Other endpoints measured (metamyelocytes, microcytosis, macrocytosis, poikilocytosis, hypochromia and polychromasia) were too rare to quantify meaningfully.

Exposure-Response Associations for Recent Benzene Exposure. Regression analyses of the blood count data on average 4-week benzene exposure are reported in Table 10. Negative associations were found between benzene exposure and counts of red blood cells, white blood cells, neutrophils and atypical lymphocytes. In addition, once potential confounders were adjusted for there was also a weak negative association for lymphocytes. A further analysis (not shown) of confounding variables in relation to lymphocyte counts suggested that smoking status may have masked the association between benzene and lymphocyte count in the unadjusted analyses.

Exposure-response analyses that included both dose and dose-squared terms did not reveal any indication of quadratic curvature in the dose-response for any of the blood counts (Table 8). Analyses were also conducted to examine blood counts at low levels of benzene exposure (≤ 0.5 ppm). As it turned out, for 4-week average exposures, all the exposed subjects ≤ 0.5 ppm were actually <0.25 ppm, so it was not possible to look at gradations of exposure in this range. Table 11 shows the comparison of the low-level exposed and unexposed groups. There were statistically significant depressions in red blood cells, white blood cells and neutrophils in the exposed group, and multiple regression analyses showed that these associations remained after control of confounding variables.

Exposure-Response Associations for Cumulative Exposure. Table 3 shows the distribution of cumulative benzene exposures, as estimated from industrial hygiene and medical records. It is notable that 27% of the exposed group had accumulated over 100 ppm-years of exposure and another 24% had 50-100 ppm-years.

Table 12 shows the associations for blood cell counts with cumulative benzene exposure (ppm-years). There were statistically significant decreases in red blood cells, white blood cells and neutrophils in relation to cumulative benzene exposure. However, for red blood cells the association seemed to be primarily a difference between the unexposed and exposed groups, with little gradation according to amount of cumulative exposure, so a causal association is suspect.

Regression analyses by cumulative benzene exposure were conducted, in order to adjust for possible confounding variables (Table 13). The adjusted analyses showed strong inverse associations of cumulative benzene exposure with red blood cells, white blood cells, neutrophils

and monocytes, plus a weaker association for eosinophils. There was a weak positive association of benzene exposure with platelets, which may be a chance finding. However, there was no statistically significant association of lymphocytes and cumulative benzene exposure.

Further analyses were conducted to determine the relative contributions of benzene exposure duration and concentration to the cumulative effects seen. Table 14 displays the blood count data according to duration of exposure, while table 15 displays it according to estimated average exposure concentration. Only red blood cells and neutrophils show associations with duration of exposure, while red blood cells, white blood cells, neutrophils and eosinophils show associations with average exposure concentration.

To compare the effects of benzene exposure duration and concentration analytically, an exposure-response regression analysis was conducted with both duration and concentration in the model. The results in Table 16 show that benzene concentration predicts depression in red blood cell, white blood cell, lymphocyte, neutrophil, monocyte and eosinophil counts, while exposure duration shows a weak association only for neutrophils. Hence, benzene concentration appears much more predictive of bone marrow depression than duration of exposure is.

Results for Albumin Adducts

For both benzene oxide adducts and 1,4-benzoquinone adducts there were strong exposure-response associations (Table 9). Regression analyses (Table 10) showed that, after adjustment for potential confounding by smoking and other variables, there was still a strong association between benzene exposure and these endpoints. Exposure-response analyses that included a dose-squared term showed convex curvature for both albumin adduct variables (Table 17). This indicates sensitivity at relatively low doses. However, at the lowest doses (<0.25 ppm) there was no positive association between benzene exposure and albumin adducts (Table 11).

An examination of cumulative benzene exposure showed strong exposure-response associations with both benzene oxide and 1,4-benzoquinone adducts (Tables 12 and 13), and an increase was evident for the lowest cumulative-dose group (mean of 16 ppm-years) in Table 12. The tabulations in tables 14 and 15 show that these adducts do not manifest a consistent increase with duration of exposure, but do with average exposure concentration. This is confirmed by the regression analyses in Table 16, in which both types of adducts show a strong exposure-response association with respect to exposure concentration but not to exposure duration.

Results for Chromosome Aberrations

The exposure-response results for various types of chromatid and chromosome aberrations are shown in Table 9. Only a few showed a statistically significant association with the 4-week average benzene exposure measure. There were moderate associations for total chromatid aberrations (breaks, deletions and exchanges), total chromosome aberrations (breaks, acentrics,

minute fragments, rings and dicentrics, but excluding gaps), total chromatid/chromosome aberrations and chromatid breaks. However, when possible confounding variables were controlled for and benzene exposure was treated as a continuous variable, the results were somewhat different (Table 10). There were moderate associations of benzene exposure with chromatid gaps and chromosome breaks, but not for any of the summary measures of chromatid or chromosome aberrations.

More detailed analyses of the exposure-response associations were conducted. When the low part (<0.5 ppm) of the exposure spectrum was examined (Table 11), there were positive associations for total chromatid aberrations, total chromosome aberrations and total chromatid/chromosome aberrations, as well as for chromatid breaks and chromosome acentrics. An analysis of a linear-quadratic dose-response model (Table 17) did not show quadratic curvature for any of the chromatid/chromosome aberration variables.

A tabulation by cumulative benzene exposure (Table 12) showed moderate to strong associations for total chromatid aberrations, total chromosome aberrations, total aberrations, chromatid breaks and acentrics. The regressions of the chromosome aberration data on cumulative benzene exposure (Table 13) showed weak to moderate associations with total chromatid aberrations, chromosome aberrations, total aberrations, chromatid gaps and breaks and chromosome breaks. Breakdowns of cumulative benzene exposure into its components – exposure duration and concentration – are shown in Tables 14 and 15. Analyses of these components (Table 16) showed that exposure duration was predictive of total chromatid aberrations, total aberrations, chromatid breaks and acentrics, while average exposure concentration was predictive of chromatid gaps and total aberrations. It is not clear whether the different attribution for the two sets of variables is meaningful or is possibly due to chance fluctuations.

Results for Chromosome Aneuploidy

The tabulations in Table 9 and the regression analyses in Table 10 show that, of the aneuploidy variables, only hypodiploidy (45 chromosomes) shows a positive association with benzene exposure. Table 11 indicates that no effects were seen at low exposure levels and Table 8 provides no indication of curvilinear effects. However, analyses by cumulative benzene exposure (Tables 12 and 13) showed associations for hypodiploidy and aneuploidy (45 or 47 chromosomes). Further analyses showed that both hypodiploidy and aneuploidy were associated with average exposure concentration but not exposure duration (Table 16).

Interactions between Benzene-Exposure Effects and Sex, Smoking and Toluene Exposure

A series of interaction analyses was performed to evaluate several possible scenarios

regarding differences in individual susceptibility or the co-acting effects of another environmental exposure besides benzene. The interaction analyses were based on multiple regression procedures. The interaction term was the product of the dichotomous sex, smoking or toluene variable times the continuous benzene exposure variable. (Toluene concentration (which was the geometric mean across 3-4 weekly measurements) was dichotomized at 20 ppm.) In the analyses the main effects for the exposure or susceptibility variables were first included in the analysis, then the interaction term with benzene was added. Other possible confounding variables were also entered into the model.

Interactions with Gender. One question of interest is whether males and females differ in the relative efficiencies of their benzene metabolic pathways, which would imply that for a given dose one gender would excrete more (or more rapidly) a particular benzene metabolite. There might then also be differences in the degree of damage caused by the metabolite (or intermediates in its pathway), which could manifest themselves in the blood or chromosome measures.

Table 18 shows there were a number of interactions of gender on the benzene effect on biomarkers. The interactions included all of the urine biomarkers and the 1,4-benzoquinone albumin adduct, but only 1 out of 23 blood/chromosome/aneuploidy variables showed a statistically significant interaction which is consistent with chance. The remarkable picture that emerges from these interactions, however, is that in all cases where there was an interaction, women were more sensitive to benzene effects than men.

Interactions with Smoking. The interactive effects of smoking were examined both across the entire exposure range and among only those with relatively low exposures (<5 ppm) (Table 19). The results are difficult to interpret because the direction of the interactions was about evenly divided between a greater effect among smokers and nonsmokers. Furthermore, there was little consistency between the results across the exposure range and the corresponding results in the lower dose range only. None of the interactive effects for blood counts or chromosome variables reached a conventional level of statistical significance ($p=.05$). We conclude that there are probably not any meaningful interactive effects of smoking and benzene exposure.

Interactions with Toluene Exposure. Toluene and benzene compete for cellular binding sites when toluene levels are high, so it was thought important to examine whether there were interactions between the two exposures in the study data. We first tabulated the outcome variables according to high/low benzene and high/low toluene simultaneously (Table 20). Then we evaluated whether there were any interactions between toluene level (dichotomized) and benzene effects in a regression analysis (Table 21). Only 5 out of 37 variables analyzed showed evidence of an interaction at a conventional level of statistical significance ($p \leq .05$), and there did not seem to be any consistent pattern of the high- or low-toluene group showing a greater effect, so we conclude there is no important interaction between benzene and toluene exposures in conferring risk. Put together with the assessment that toluene did not serve as a confounder of benzene effects (see above and Tables 6 and 10), we do not believe that toluene exposure played an appreciable role in this study.

Additional Methodological Analyses to Check on Assumptions

Table 21 shows two sets of analyses to check on assumptions. Although it seems natural to have a zero dose point in the study, there may be concerns over the comparability of the control (unexposed) and exposed groups. We took pains to try to obtain a comparable control group, sampling so that they were similar to the exposed group with respect to sex, smoking proportions and performing semi-skilled work at a factory in the same geographic area. Further, all assays were performed in a blinded fashion with exposed and unexposed subjects intermixed. However, given that the exposed and unexposed workers were employed at different factories making different kinds of products, the assumption that the exposed and unexposed group are from the same population should be examined. We therefore conducted a set of exposure-response analyses for the more important outcome variables that also included an indicator variable for exposure group, in order to determine the degree to which differences in baseline rates between the exposed and unexposed may have distorted the results. The results are shown in the second column of Table 22 and can be compared to the regression results in tables 6 and 10. The exposure-response coefficients tended to be somewhat smaller when the group indicator variable was included, but this is to be expected because of shared variance between the two coefficients. There was no evidence of marked differences between the results in Table 22 and those in Tables 6 and 10 that would cause concern regarding disparities between the exposed and unexposed groups.

If, indeed, the unexposed group was appreciably different from the exposed group(s) in their baseline risk of the biomarker endpoints under study, one would expect to see this effect most sharply in the dose-response analyses that examined both linear and quadratic terms in dose, since a baseline difference would tend to induce nonlinearity (if the curve was linear among the exposed subgroups). We therefore performed more detailed analyses of the linear-quadratic relationships of benzene with the biomarker outcomes (Tables 8 and 17).

For the albumin adduct and chromosome aberration analyses (Table 17) there were no meaningful differences between analyses when the unexposed group was included vs. excluded. However, for the blood counts there were some differences. For red blood cells and neutrophils both the linear and quadratic terms became less statistically significant. It is not clear how much of these changes are due to loss of statistical power (because excluding the unexposed group produces a smaller sample size) and how much are due to confounding by some unknown variable in the unexposed group.

For the urine metabolites we saw no meaningful differences between analyses including or excluding the unexposed group (Table 8). In addition, to evaluate the meaning of the statistically significant negative quadratic coefficients (which imply a greater effect per unit dose at low doses than at high doses) for the urine metabolites, we examined whether this might be due to the choice of scale (i.e., we had taken the logarithms of the metabolite variables in almost all cases) which might induce this curvilinearity. Analyses performed on the raw urine metabolite variables (Table 8) also tended to show negative curvilinearity, suggesting it was not primarily a scaling artifact.

Again, there was no evidence that including vs. excluding the control group had a substantial impact on the results.

In summary, the bulk of the evidence does not indicate that including the unexposed group in the analyses introduces confounding when analyses have been properly controlled for sex, age and smoking.

Another set of methodological analyses was conducted because the exposure data and a number of the outcome variables were right skewed. Semi-parametric regression analyses based on exposure ranks and outcome-variable ranks (Conover, 1981) were undertaken to see if they corroborated the parametric analyses (Table 22, last column). In general, the correspondence between the results in table 22 (semi-parametric) and in tables 6 and 10 (parametric) was excellent, with substantial disparities for only 3 variables (2 of which – total chromatid aberrations and total aberrations – were highly correlated so they perhaps should be counted as only a single disparity).

Correlations among Biomarkers

As shown in Table 23, the urine biomarkers correlated substantially with each other. T,t-MA and S-PMA after work correlated 0.85, while they correlated with phenol 0.41 and 0.47 respectively. The albumin adduct biomarkers also correlated substantially with the urine biomarkers, especially with S-PMA. Benzene oxide adducts and 1,4-benzoquinone adducts correlated 0.74 with each other. The correlations of the blood cell counts and chromosome aberrations with each other were quite low, as were their correlations with the urine or adduct biomarkers.

Susceptibility to Chromosome Damage by Benzene

A question of interest is whether persons who show a relatively great amount of toxicity or damage to the hematopoietic system for a given benzene dose -- as indexed by selected biomarkers (t,t-MA and albumin adducts) or by indicators of bone marrow depression -- also show more damage to chromosomes. To evaluate this, we calculated individuals' residuals from their predicted levels of biomarkers or bone marrow depression, based on the regression on benzene exposure, i.e., we determined how much they fell above or below the regression line. Their score as to how much they fell above or below the regression line was then correlated with the chromosome aberration/aneuploidy data to see if there were associations.

Table 24 shows the results of these analyses. A positive correlation indicates that persons with more toxicity or hematopoietic damage also show more chromosome damage. There are only a few statistically significant associations and these do not seem to show any consistent pattern. How to interpret these results is unclear. It may be that this approach to indexing biological damage is too crude or simplistic, or that genotypic differences will be more predictive

than phenotypic ones. In spite of these largely negative results, we believe the question merits more exploration in the future.

We also performed a set of multiple regression analyses to determine whether the chromosome aberration and aneuploidy effects of benzene might be accentuated among those who had depressed hematopoiesis, as indexed by the red blood cell counts and the neutrophil counts, the two cell types that showed the strongest associations with benzene exposure in this study. Analyses of the chromosome aberration and aneuploidy endpoint were undertaken which included the unexposed group plus the benzene-exposed either with RBC below 400 (which was the 18th percentile in the unexposed group and the 49th percentile in the exposed group) or with neutrophil counts below 3000 (which was the 17th percentile in the unexposed group and the 48th percentile in the exposed group). The results of this were uninformative; there were no significant differences in the slope of the regression line for those with low RBC or neutrophils as opposed to those with higher levels (data not shown).

Finally, a set of analyses was performed to see if levels of the benzene urine metabolites were predictive of depressed hematopoiesis above and beyond the amount of depression predicted by benzene exposure itself. The urine metabolite variables chosen for possible inclusion in the regression models were t,t-MA, S-PMA and Phenol (all as measured at the end of the workday). For red blood cells, white blood cells, neutrophils, monocytes, basophils and platelets none of the urine metabolites were predictive (all $p>0.10$). However, there were several associations ($p<0.05$). High t,t-MA excretion was protective with regard to lymphocytes, but was predictive of a greater mean corpuscular volume and of more 1,4-benzoquinone adducts. High S-PMA was predictive of decreased eosinophils, elevated benzene oxide adducts and elevated 1,4-benzoquinone adducts, while high phenol was predictive of depressed hematocrit.

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NYU-HEI Benzene Study: Tables Presenting the Statistical Analyses of the Combined Year-1 and Year-2 Data on Benzene and Biomarkers

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Table 1. Comparison of Biomarkers for the Year-1 and Year-2 Unexposed Control Groups

Variable	Year 1 Controls	Year 2 Controls
Number	25	26
Age	31.7 ± 9.3	34.9 ± 4.8
Sex (% female)	52	54
Smoking % (reported + high cotinine)	32	31
t,t-MA (mg/g cr.), pre-work	.48 ± .71	.21 ± .37 [A]
t,t-MA (mg/g cr.), post-work	.31 ± .30	.22 ± .24
t,t-MA (mg/g cr.), post – pre	-.17 ± .77	.01 ± .43
S-PMA (µg/g cr.), pre-work	1.24 ± 2.3	2.35 ± 5.6
S-PMA (µg/g cr.), post-work [C]	.88 ± 1.1	2.83 ± 1.9 [C]
S-PMA (µg/g cr.), post – pre	-.36 ± 2.2	.48 ± 5.9
Phenol (mg/g cr.), pre-work	16.4 ± 16	7.2± 24 [A]
Phenol (mg/g cr.), post-work [C]	11.9 ± 12	2.0 ± 4
Phenol (mg/g cr.), post – pre	-4.5 ± 16	-5.2 ± 25
Cotinine (µg/g cr.)	560 ± 1041	559 ± 1220
Red blood cells ($x10^{10}$/L)	469 ± 63	457 ± 40
White blood cells ($x10^{6}$/L)	6752 ± 1337	6592 ± 1669
Lymphocytes ($x10^{6}$/L)	2354 ± 789	2062 ± 776
Neutrophils ($x10^{6}$/L)	3893 ± 1061	4113 ± 1162
Monocytes ($x10^{6}$/L)	286 ± 123	249 ± 152
Eosinophils ($x10^{6}$/L)	165 ± 212	126 ± 94
Basophils ($x10^{6}$/L)	10.5 ± 23.9	8.1 ± 13.9
Bands	35.6 ± 51.5	29.8 ± 36.4
Ct aberrations (breaks, deletions, exchanges)	1.12 ± 1.39	1.27 ± 1.34
Total Cs aberrations (except gaps)	.68 ± 1.65	.50 ± .71
Total aberrations (except gaps)	1.80 ± 2.50	1.77 ± 1.56
Chromatid gaps [B]	1.96 ± 1.93	.81 ± 1.20
Chromatid breaks	1.12 ± 1.39	1.27 ± 1.34
Chromosome breaks	.28 ± .61	.12 ± .33

Variable	Year 1 Controls	Year 2 Controls
Number	25	26
Acentrics	.40 ± 1.23	.38 ± .70
Hypodiploidy - 45 chrom.	.32 ± .63	.12 ± .43
Hyperdiploidy (47 chrom.)	.08 ± .28	.04 ± .20
Aneuploidy (45, 47 chrom)	1.76 ± 1.74	4.15 ± 2.94
Benzene oxide albumin adducts (nmol/g) [A]	.19 ± .10	.17 ± .06
1,4-Benzoquinone albumin adducts (nmol/g) [A]	1.32 ± .44	.96 ± .52

[A] $p \leq 0.05$

[B] $p \leq 0.01$

[C] $p \leq 0.001$

[D] The means and standard deviations reported are for the raw variables, but for the urine metabolites, the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

Table 2. Frequency Distributions and Summary Statistics of Benzene and Toluene Exposure Levels

Exposure (ppm)	Benzene, 4-week average - Number (%)	Benzene, day of urine samples - Number (%)	Toluene, day of urine samples - Number (%)
0	51	51	51
>0 - 0.5	16 (12) [A]	17 (13) [A-]	1 (1) [A]
>0.5 - 1	0	8 (6)	4 (3)
>1 - <3	32 (25)	35 (27)	13 (10)
3 - <5	25 (19)	22 (17)	11 (8)
5 - <10	22 (17)	17 (13)	31 (24)
10 - <15	11 (8)	9 (7)	12 (9)
15 - <30	8 (6)	8 (6)	32 (25)
≥ 30	16 (12)	14 (11)	26 (20)
Mean ± Std. Dev. [A]	11.1 ± 21.8	9.7 ± 16.6	26.3 ± 44.2
Median [A]	3.8	3.2	12.6

[A] Percent of exposed group, or statistic based on exposed group.

Table 3. Frequency Distribution of "Lifetime" Cumulative Benzene Exposures in the Exposed Group

Cumulative Exposures (ppm-years)	Number (Percent)
<20	19 (15)
20-49	46 (35)
50-99	31 (24)
100-149	19 (15)
150-249	9 (7)
250-630	6 (5)

Table 4. Means and Standard Deviations (or Percents) for Urinary, Blood and Chromosome Variables by Benzene Exposure Group

Variable	Unexposed Group (Mean ± S.D.) [D]	Exposed Group (Mean ± S.D.)
Number [E]	51	130
Sex (% female)	53%	52%
Smoking (Self-report) (%)	20%	30%
Smoking (Self-report + Cotinine >100) (%)	31%	38%
Age (years)	33.3 ± 7.4	36.3 ± 7.6
t,t-MA (mg/g cr.), pre-work [D,C]	.34 ± .58	2.05 ± 3.75
t,t-MA (mg/g cr.), post-work [C]	.26 ± .27	6.16 ± 6.48
t,t-MA (mg/g cr.), post - pre [C]	-.08 ± .62	4.11 ± 5.06
S-PMA (µg/g cr.), pre-work [C]	1.8 ± 4.3	82.2 ± 147.4
S-PMA (µg/g cr.), post-work [C]	1.9 ± 1.9	285.4 ± 644.1
S-PMA (µg/g cr.), post - pre [C]	.07 ± 4.45	203.20 ± 569.20
Phenol (mg/g cr.), pre-work	11.7 ± 21.1	14.6 ± 34.8
Phenol (mg/g cr.), post-work [C]	6.9 ± 10.0	30.4 ± 55.9
Phenol (mg/g cr.), post - pre [A]	-4.9 ± 20.8	15.9 ±59.7
Cotinine (µg/g cr.)	560 ± 1125	628 ± 1177
Red blood cells (x10^{10}/L) [C]	463.0 ± 52.4	400.4 ± 56.5
Hematocrit	44.2 ± 5.3	42.9 ± 4.0
Platelets (x10^9/L)	277 ± 43	280 ± 67
White blood cells (x10^6/L) [A]	6671 ± 1502	6099 ± 1431
Lymphocytes (x10^6/L)	2205 ± 789	2300 ± 744
Neutrophils (x10^6/L) [C]	4006 ± 1108	3360 ± 1057
Monocytes (x10^6/L)	267 ± 139	279 ± 146
Eosinophils (x10^6/L)	145 ± 162	119 ± 124
Basophils (x10^6/L)	9.3 ± 19.3	7.6 ± 14.1
Atypical lymphocytes	.10 ± .22	.16 ± .34
Bands	32.6 ± 44.1	30.1 ± 39.4
Ct aberrations (breaks, deletions, exchanges) [C]	1.20 ± 1.36	2.01 ± 1.53

Total Cs aberrations (except gaps) [A]	0.59 ± 1.25	.96 ± 1.16
Total aberrations (except gaps) [C]	1.78 ± 2.05	2.97 ± 2.11
Chromatid gaps [B]	1.37 ± 1.66	.86 ± 1.48
Chromatid breaks [C]	1.20 ± 1.36	1.99 ± 1.51
Chromosome breaks	.20 ± .49	.29 ± .58
Acentrics [A]	.39 ± .98	.62 ± .89
Hypodiploidy - 45 chrom.	.22 ± .54	.23 ± .57
Hypodiploidy - <45 chrom. [C]	2.71 ± 2.69	6.62 ± 7.41
Hyperdiploidy - 47 chrom.	.06 ± .24	.13 ± .43
Aneuploidy - 45/47 chrom	.27 ± .57	.36 ± .72
Aneuploidy - 44,45,47 chrom [C]	2.98 ± 2.69	6.98 ± 7.43
Benzene oxide albumin adducts (nmol/g) [C]	.18 ± .08	.57 ± .75
1,4-Benzoquinone albumin adducts (nmol/g) [C]	1.14 ± .51	2.96 ± 2.14

[A] $p \le 0.05$

[B] $p \le 0.01$

[C] $p \le 0.001$

[D] The means and standard deviations reported are for the raw variables, but for the urine metabolites, the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

[E] Maximum number -- some variables were missing for a small number of subjects. Hematocrit and Platelets were assessed only for year 2: 26 unexposed and 105 exposed.

Table 5. Means and Standard Deviations (or Percents) for Urine Variables, Grouped According to **Benzene Exposure** on the Day of the the Urine Collection

Variable	Unexposed	≤ 1 ppm	>1 - 5 ppm	>5 - 15 ppm	>15-30 ppm	>30 ppm
No. of subjects	51	25	57	26	8	14
Female (%)	53	76	44	38	75	57
Smokers (%; reported + cotinine)	31	12	49	42	38	36
Mean Age	33.3 ± 7.4	36.5 ± 4.3	36.8 ± 7.9	35.6 ± 9.5	37.4 ± 6.5	34.4 ± 8.1
Mean benzene exposure (the day of the urine samples)	0	.34 ± .32	2.56 ± 1.10	9.12 ± 3.37	22.6 ± 4.0	49.1 ± 23.2
t,t-MA (mg/g cr.), pre-work [C,D]	.34 ± .58	.61 ± .77	1.06 ± .68	2.48 ± 3.52	6.31 ± 7.57	5.39 ± 7.03
t,t-MA (mg/g cr.), post-work [C]	.26 ± .27	.96 ± 1.16	4.12 ± 3.39	9.33 ± 6.29	13.45 ± 10.13	13.70 ± 7.03
t,t-MA (mg/g cr.), post - pre [C]	-.08 ±. .62	.35 ± .73	3.06 ± 3.14	6.85 ± 5.60	7.13 ± 8.97	8.30 ± 5.97
S-PMA (μg/g cr.), pre-work [C]	1.80 ± 4.3	31.5 ± 62.3	52.6 ± 63.4	146 ± 255	196 ± 222	109 ± 118
S-PMA (μg/g cr.), post-work [C]	1.87 ± 1.86	23.9 ± 37.9	117 ± 143	464 ± 608	580 ± 872	938 ± 1441
S-PMA (μg/g cr.), post - pre [C]	.07 ± 4.45	-7.7 ± 32	65 ± 119	317 ± 366	384 ± 868	829 ± 1360
Phenol (mg/g cr.), pre-work [B]	11.7 ± 21.1	4.5 ± 14.2	7.5 ± 9.7	26.9 ± 54.9	60.0 ± 83.7	15.8 ± 16.2
Phenol (mg/g cr.), post-work [C]	6.9 ± 10.0	4.9 ± 7.3	13.4 ± 12.9	32.2 ± 36.6	52.8 ± 74.4	128 ± 107
Phenol (mg/g cr.), post - pre [C]	-4.85 ± 20.76	.39 ± 15.30	5.90 ± 13.85	5.33 ± 67.20	-2.05 ± 63.37	113 ± 109
Cotinine (μg/g cr.)	560 ± 1124	115 ± 270	786 ± 1216	901 ± 1597	384 ± 658	527 ± 1134

[A] $p \leq 0.05$, test for exposure-response trend
[B] $p \leq 0.01$, test for exposure-response trend
[C] $p \leq 0.001$, test for exposure-response trend
[D] For the urinary metabolites, the means and standard deviations reported are the raw variables, but the statistical tests were performed on the log transformed data.

Table 6. Regression Analyses of Urine Biomarkers by **Benzene Exposure Level** (Day of Urine Collection), With and Without Adjustment for Sex, Age, Cotinine, and Toluene Exposure Level [E]

Variable	Benzene Regress. Coeff. ± S.E., Unadjusted	Benzene Regress. Coeff. ± S.E., Adjusted for Sex, Age, Cotinine	Benzene Regress. Coeff. ± S.E., Also Adjusted for Toluene
t,t-MA (mg/g cr.), pre-work (log)	.027 ± .003 [D]	.027 ± .003 [D]	.026 ± .003 [D]
t,t-MA (mg/g cr.), post-work (log)	.046 ± .004 [D]	.046 ± .004 [D]	.041 ± .004 [D]
t,t-MA (mg/g cr.), post - pre (log)	.019 ± .004 [D]	.019 ± .004 [D]	.016 ± .003 [D]
S-PMA (µg/g cr.), pre-work (log)	.058 ± .010 [D]	.058 ± .010 [D]	.047 ± .009 [D]
S-PMA (µg/g cr.), post-work (log)	.095 ± .010 [D]	.096 ± .010 [D]	.081 ± .008 [D]
S-PMA (µg/g cr.), post - pre (log)	.037 ± .006 [D]	.037 ± .006 [D]	.0.034 ± .006 [D]
Phenol (mg/g cr.), pre-work (log)	.021 ± .008 [B]	.021 ± .008 [B]	.023 ± .008 [B]
Phenol (mg/g cr.), post-work (log)	.067 ± .007 [D]	.067 ± .007 [D]	.067 ± .007 [D]
Phenol (mg/g cr.), post – pre	2.35 ± .245 [D]	2.34 ± .247 [D]	2.35 ± .251 [D]
t,t-MA/S-PMA ratio (log)	-.003 ± .0009 [B]	-.003 ± .0009 [B]	-.002 ± .0009 [A]

[A] $p \leq 0.05$, test for exposure-response trend
[B] $p \leq 0.01$, test for exposure-response trend
[C] $p \leq 0.001$, test for exposure-response trend
[D] $p \leq 0.0001$, test for exposure-response trend
[E] The controls were included in the analysis and were randomly assigned values between 0 and 0.01 ppm which was the approximate lower limit of detection.

Table 7. Urine Biomarkers for **Low-Exposure Subjects (≤1 ppm)**: Benzene Exposure-Response Trend and Comparison of Unexposed vs. >0-0.5 ppm Groups

Variable [D]	Unexposed	>0 - 0.5 ppm	>0.5 - 1 ppm	p-Trend [A]	p (0 vs. ≤0.5 ppm)
No. of subjects	51	17	8		
Female (%)	53%	94%	38%		
Smokers (%; reported + cotinine))	31%	6%	25%		
Mean benzene exposure (day of urine)	0	.14 ± .11	.76 ± .17		
t,t-MA (mg/g cr.), pre-work	.34 ± .58	.26 ± .54	1.36 ± .65	<.0001	--
t,t-MA (mg/g cr.), post-work	.26 ± .27	.36 ± .34	2.25 ± 1.24	<.0001	--
t,t-MA (mg/g cr.), post - pre	-.08 ± .62	.10 ± .51	.89 ± .87	.006	--
S-PMA (µg/g cr.), pre-work	1.80 ± 4.3	7.30 ± 22	83.0 ± 88.2	<.0001	--
S-PMA (µg/g cr.), post-work	1.87 ± 1.86	6.49 ± 11.3	60.8 ± 48.4	<.0001	.001
S-PMA (µg/g cr.), post - pre	.07 ± 4.45	-.81 ± 10.9	-22.2 ± 52.9	--	--
Phenol (mg/g cr.), pre-work	11.7 ± 21	1.22 ± 2.81	11.5 ± 24	--	--
Phenol (mg/g cr.), post-work	6.86 ± 10	3.25 ± 4.8	8.44 ± 10.5	--	--
Phenol (mg/g cr.), post - pre	-4.85 ± 21	2.02 ± 5.8	-3.09 ± 27	--	--
Cotinine (µg/g cr.)	560 ± 1124	64 ± 180	222 ± 396	--	(.01)

[A] Multiple regression analysis controlling for sex, age, cotinine and toluene exposure.

Table 8. **Linear-Quadratic** Model: Shape of the Exposure-Response Relationship for Urine Metabolites [A,B]

	Including Unexposed Group, Log-transformed Dependent Variables		Excluding Unexposed Group, Log-transformed Dependent Variables		Including Unexposed Group, Raw Dependent Variables	
Dependent Variable	Linear term	Quadratic term	Linear term	Quadratic term	Linear term	Quadratic term
t,t-MA, pre-work	.057 ± .008 (<.0001)	-.059 ± .014 (.0001)	.05 ± .009 (<.0001)	-.04 ± .02 (.008)	.19 ± .04 (<.0001)	-.12 ± .08 (--)
t,t-MA, post-work	.12 ± .01 (<.0001)	-.15 ± .02 (<.0001)	.09 ± .01 (<.0001)	-.10 ± .02 (<.0001)	.65 ± .07 (<.0001)	-.69 ± .12 (<.0001)
t,t-MA, post – pre	.06 ± .009 (<.0001)	-.09 ± .02 (<.0001)	.04 ± .01 (.0002)	-.06 ± .02 (.003)	.46 ± .06 (<.0001)	-.57 ± .11 (<.0001)
S-PMA, pre-work	.18 ± .02 (<.0001)	-.24 ± .05 (<.0001)	.10 ± .03 (.0001)	-.13 ± .05 (.005)	10 ± 1.9 (<.0001)	-15 ± 3.5 (<.0001)
S-PMA, post-work	.27 ± .03 (<.0001)	-.35 ± .05 (<.0001)	.18 ± .02 (<.0001)	-.22 ± .04 (<.0001)	48 ± 7.7 (<.0001)	-59 ± 14 (.0001)
S-PMA, post – pre	.09 ± .02 (<.0001)	-.11 ± .03 (.0001)	.08 ± .02 (<.0001)	-.09 ± .03 (.006)	38 ± 6.9 (<.0001)	-44 ± 13 (.0007)
Phenol, pre-work	.10 ± .02 (<.0001)	-.15 ± .04 (.0003)	.12 ± .02 (<.0001)	-.17 ± .04 (<.0001)	2.0 ± .48 (<.0001)	-3.3 ± .88 (.0002)
Phenol, post-work	.13 ± .02 (<.0001)	-.12 ± .04 (.002)	.12 ± .02 (<.0001)	-.11 ± .04 (.007)	2.4 ± .54 (<.0001)	.56 ± .98 (--)
Phenol, post – pre	.48 ± .66 (--)	3.7 ± 1.2 (.003)	.24 ± .84 (--)	4.0 ± 1.5 (.009)	.48 ± .65 (--)	3.7 ± 1.2 (.003)

[A] P-values are given only if the p-value is ≤ 0.10. Quadratic coefficients were based on ((benzene exposure in ppm)/10)^2. All analyses controlled for age, sex and cotinine.

[B] The controls were randomly assigned values between 0 and 0.01 ppm which was the approximate lower limit of detection.

Table 9. Means and Standard Deviations (or Percents) for Blood Count, Chromosome Aberration, Chromosome Ploidy and Albumin Adduct Variables, Grouped According to **Benzene Exposure** (Averaged over Four Weeks)

Variable [D]	Unexposed	>0 - 5 ppm	>5 - 15 ppm	>15-30 ppm	>30 ppm
No. of subjects	51	73	33	8	16
Female (%)	53	55	33	88	63
Smokers (%; reported + cotinine))	31	36	55	0	38
Mean Age	33.3 ± 7.4	36.6 ± 6.4	36.3 ± 9.6	38.5 ± 8.5	33.4 ± 7.7
Mean benzene exposure (averaged over 4 weeks)	.004 ± .003	2.26 ± 1.35	8.67 ± 2.44	19.9 ± 3.1	51.8 ± 43.3
Red blood cells ($x10^{10}$/L) [C]	463 ± 52	399 ± 59	410 ± 60	387 ± 18	392 ± 50
Hematocrit (year 2 only)	44 ± 5.3	43 ± 3.5	43 ± 5.3	41 ± 3.6	41 ± 1.4
Platelets ($x10^9$/L; year 2 only)	277 ± 43	280 ± 62	285 ± 82	259 ± 63	278 ± 20
White blood cells ($x10^6$/L) [C]	6671 ± 1502	6415 ± 1266	6006 ± 1752	5825 ± 1550	4988 ± 615
Lymphocytes ($x10^6$/L)	2205 ± 789	2429 ± 741	2226 ± 691	2248 ± 1152	1890 ± 453
Neutrophils ($x10^6$/L) [C]	4006 ± 1108	3541 ± 944	3315 ± 1408	3116 ± 610	2753 ± 580
Monocytes ($x10^6$/L)	267 ± 139	292 ± 144	270 ± 113	315 ± 270	220 ± 131
Eosinophils ($x10^6$/L)	145 ± 162	110 ± 96	159 ± 189	87 ± 72	93 ± 66
Basophils ($x10^6$/L)	9.3 ± 19.3	7.7 ± 13.6	8.2 ± 14.9	14.5 ± 23.5	2.2 ± 6.0
Bands	32.6 ± 44.1	33.4 ± 40.5	22.7 ± 36.5	42.3 ± 36.4	24.0 ± 41.6
Atypical lymphocytes	.10 ± .22	.24 ± .41	.10 ± .23	.04 ± .12	0
Ct breaks, deletions, exchanges [B]	1.20 ± 1.4	2.01 ± 1.5	2.00 ± 1.4	1.43 ± 1.3	2.25 ± 1.8
Total Cs aberrations (except gaps) [A]	0.59 ± 1.3	.97 ± 1.2	.74 ± 1.0	.71 ± 1.3	1.44 ± 1.3
Total aberrations (except gaps) [B]	1.78 ± 2.1	2.99 ± 2.1	2.74 ± 2.0	2.14 ± 2.1	3.69 ± 2.5

Variable [D]	Unexposed	>0 - 5 ppm	>5 - 15 ppm	>15-30 ppm	>30 ppm
Chromatid gaps	1.37 ± 1.7	.59 ± 1.0	.71 ± 1.8	1.00 ± 1.0	2.31 ± 2.0
Chromatid breaks [B]	1.20 ± 1.4	1.99 ± 1.5	2.00 ± 1.4	1.43 ± 1.3	2.25 ± 1.8
Chromosome breaks	.20 ± .50	.25 ± .55	.23 ± .56	.57 ± .98	.50 ± .52
Acentrics	.39 ± .98	.70 ± .98	.48 ± .63	.14 ± .38	.75 ± 1.0
Hypodiploidy - 45 chrom.	.22 ± .54	.12 ± .33	.39 ± .84	.29 ± .49	.38 ± .72
Hypodiploidy - ≤ 44 chrom.	2.7 ± 2.7	7.0 ± 7.1	7.6 ± 9.6	5.5 ± 5.2	3.5 ± 3.1
Hyperdiploidy	.06 ± .24	.16 ± .50	.13 ± .34	0	.06 ± .25
Aneuploidy (45 or 47 chrom.)	.27 ± .57	.29 ± .61	.52 ± .96	.29 ± .49	.44 ± .73
Total aneuploidy (≤45, 47 chrom.)	3.0 ± 2.7	7.3 ± 7.2	8.1 ± 9.5	5.8 ± 5.1	3.9 ± 3.5
Stimulated cells/1000 cells	568 ± 126	581 ± 136	562 ± 132	579 ± 79	616 ± 137
Metaphases/1000 cells [B]	45 ± 26	49 ± 25	47 ± 20	45 ± 22	72 ± 27
Benzene oxide albumin adducts (nmol/g) [C]	.18 ± .08	.31 ± .13	.60 ± .38	.87 ± .46	1.50 ± 1.73
1,4-Benzoquinone albumin adducts (nmol/g) [C]	1.1 ± .5	1.8 ± 1.2	3.7 ± 1.9	3.3 ± 1.7	6.3 ± 1..9

[A] $p \leq 0.05$, test for exposure-response trend

[B] $p \leq 0.01$, test for exposure-response trend

[C] $p \leq 0.001$, test for exposure-response trend

[D] The means and standard deviations reported are for the raw variables, but for the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

Table 10. **Benzene Exposure-Response** Regression Analyses of Blood Counts, Chromosome Aberrations and Albumin Adducts by Benzene Exposure Level (4-week Average), With and Without Adjustment for Sex, Age, Toluene and Cotinine Levels [E,F]

Variable	Benzene Regress. Coeff. ± S.E., Unadjusted, All Subjects	Benzene Regress. Coeff. ± S.E., Adjusted, All Subjects
Red blood cells ($x10^{10}$/L)	-1.01 ± .37 [B]	-.60 ± .31 [A]
Hematocrit	-.061 ± .055	-.062 ± .054
Platelets ($x10^{9}$/L)	-.164 ± .80	-.47 ± .92
White blood cells ($x10^{6}$/L)	-33.4 ± 8.5 [C]	-32.6 ± 8.8 [C]
Lymphocytes ($x10^{6}$/L)	-7.8 ± 4.5	-9.0 ± 4.6 [A]
Neutrophils ($x10^{6}$/L)	-23.6 ± 6.5 [C]	-21.6 ± 6.6 [B]
Monocytes, log($x10^{6}$/L)	-.005 ± .003	-.006 ± .003
Eosinophils, log($x10^{6}$/L)	-.005 ± .007	-.002 ± .007
Basophils, log($x10^{6}$/L)	-.003 ± .009	-.006 ± .009
Bands (√)	-.025 ±.021	-.023 ± .022
Atypical lymphocytes (√)	-.004 ± .002 [A]	-.004 ± .002 [A]
Ct breaks, deletions, exchanges (√)	.004 ± .003	.002 ± .003
Total Cs aberrations (except gaps) (√)	.004 ± .003	.000 ± .002
Total aberrations (except gaps) (√)	.006 ± .004	.004 ± .004
Chromatid gaps (√)	.008 ± .003 [A]	.010 ± .003 [B]
Chromatid breaks (√)	.004 ± .003	.002 ± .003
Chromosome breaks (√)	.004 ± .002 [A]	.004 ± .002 [A]
Acentrics (√)	.001 ± .002	.0007 ± .002
Hypodiploidy - 45 chrom. (√)	.003 ± .002 [A]	.003 ± .002 [A]
Hypodiploidy - ≤ 44 chrom. (√)	.001 ± .007	-.003 ± .007
Hyperdiploidy (√)	-.0008 ± .001	-.0009 ± .001
Aneuploidy (45 or 47 chrom.) (√)	.002 ± .002	.002 ± .002
Total aneuploidy (≤45, 47 chrom.) (√)	.002 ± .007	-.002 ± .007
Stimulated cells/1000 cells	.94 ±.78	1.13 ± .80
Metaphases/1000 cells	.50 ± .15 [B]	.50 ± .15 [B]
Benzene oxide adducts, (log nmol/g)	.014 ± .001 [D]	.014 ± .001 [D]

Variable	Benzene Regress. Coeff. ± S.E., Unadjusted, All Subjects	Benzene Regress. Coeff. ± S.E., Adjusted, All Subjects
1,4-Benzoquinone adducts, (log nmol/g)	.027 ± .002 [D]	.026 ± .002 [D]

[A] $p \leq 0.05$, test for exposure-response trend
[B] $p \leq 0.01$, test for exposure-response trend
[C] $p \leq 0.001$, test for exposure-response trend
[D] $p \leq 0.0001$, test for exposure-response trend
[E] The logarithms of cotinine and toluene were used in the analysis.
[F] The controls were included in the analysis and were randomly assigned values between 0 and 0.01 ppm which was the approximate lower limit of detection.

Table 11. Blood Counts, Chromosome Aberrations and Albumin Adducts for the Unexposed Group vs. the **Exposed Group with ≤ 0.5 ppm** (4-week average) [C]

Variable	Unexposed [B] Mean ± SD	>0 to 0.5 ppm Mean ± SD	p-value [A]
No. of subjects	51	16	
Female (%)	53%	100%	
Smokers (%; reported + cotinine))	31%	0%	
Mean Age	33.3 ± 7.4	36.2 ± 3.2	
Mean benzene exposure (averaged over 4 weeks)	0	.14 ± .04	
Red blood cells ($x10^{10}$/L) [C]	463 ± 52	393 ± 49	.0006
Hematocrit	44.2 ± 5.3	43.1 ± 2.6	--
Platelets ($x10^9$/L)	277 ± 43	286 ± 71	--
White blood cells ($x10^6$/L)	6671 ± 1502	5700 ± 1226	.02
Lymphocytes ($x10^6$/L)	2205 ± 789	2015 ± 450	--
Neutrophils ($x10^6$/L)	4006 ± 1108	3254 ± 901	.02
Monocytes ($x10^6$/L)	267 ± 139	251 ± 108	--
Eosinophils ($x10^6$/L)	145 ± 162	136 ± 133	--
Basophils ($x10^6$/L)	9.3 ± 19.3	10.1 ± 14.6	--
Bands	32.6 ± 44.1	33.5 ± 40.3	--
Atypical lymphocytes	.10 ± .22	.04 ± .11	--
Ct breaks, deletions, exchanges	1.20 ± 1.36	2.19 ± 1.38	.01
Total Cs aberrations (except gaps)	0.59 ± 1.25	1.44 ± 1.41	.008
Total aberrations (except gaps)	1.78 ± 2.05	3.63 ± 2.09	.001
Chromatid gaps	1.37 ± 1.66	.63 ± .81	.09
Chromatid breaks	1.20 ± 1.36	2.19 ± 1.38	.01
Chromosome breaks	.20 ± .49	.38 ± .50	--
Acentrics	.39 ± .98	1.06 ± 1.29	.01
Hypodiploidy - 45 chrom.	.22 ± .54	.19 ± .40	--
Hypodiploidy - ≤ 44 chrom.	2.71 ± 2.69	3.00 ± 4.24	--
Hyperdiploidy	.06 ± .24	.06 ± .25	--
Aneuploidy (45 or 47 chrom.)	.27 ± .57	.25 ± .45	--

Variable	Unexposed [B] Mean ± SD	>0 to 0.5 ppm Mean ± SD	p-value [A]
Total aneuploidy (≤45, 47 chrom.)	2.98 ± 2.69	3.25 ± 4.5	--
Stimulated cells/1000 cells	568 ± 126	592 ± 132	--
Metaphases/1000 cells	45 ± 26	38 ± 21	--
Benzene oxide adducts (nmol/g)	.18 ± .08	.22 ± .13	--
1,4-Benzoquinone adducts (nmol/g)	1.14 ± .5	1.06 ± 1.9	.05

[A] Multiple regression analysis controlling for sex, age, year, smoking, cotinine and toluene exposure.

[B] The means and standard deviations reported are for the raw variables, but for the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

[C] As it turned out, all of the mean exposure levels were actually <0.25 ppm.

Table 12. Means and Standard Deviations (or Percents) for Blood Count, Chromosome Aberration and Ploidy, and Albumin Adduct Variables, Grouped According to **Lifetime Cumulative Occupational Benzene** Exposure (ppm-years)

Variable [E]	Unexposed	.1 - <30 ppm-y	30 - <50 ppm-y	50 - <100 ppm-y	≥100 ppm-y
No. of subjects	51	30	35	31	34
Female (%)	53%	30%	74%	58%	44%
Smokers (%; reported + cotinine)	31%	53%	29%	36%	38%
Mean Age	33.3 ± 7.4	33.1 ± 9.5	37.7 ± 3.2	38.7 ± 4.8	35.3 ± 9.9
Mean cumulative benzene exposure (ppm-years)	0	16.0 ± 8.0	40.8 ± 6.0	73.9 ± 14.4	187 ± 117
Current benzene exposure (mean over 4 weeks) (ppm)	.004 ± .003	3.82 ± 2.8	2.67 ± 2.7	8.85 ± 11.0	28.12 ± 36.2
Red blood cells ($x10^{10}$/L) [C]	463 ± 52	409 ± 68	391 ± 55	403 ± 53	400 ± 50
Hematocrit (year 2 only)	44.2 ± 5.3	43.4 ± 3.5	41.8 ± 3.6	43.4 ± 3.9	43.8 ± 6.1
Platelets ($x10^9$/L; year 2 only)	277 ± 43	289 ± 71	255 ± 56	288 ± 57	314 ± 85
White blood cells ($x10^6$/L) [C]	6671 ± 1502	6843 ± 1391	5943 ± 1163	6074 ± 1176	5626 ± 1701
Lymphocytes ($x10^6$/L)	2205 ± 789	2630 ± 949	2264 ± 665	2301 ± 489	2046 ± 728
Neutrophils ($x10^6$/L) [D]	4006 ± 1108	3757 ± 969	3202 ± 837	3329 ± 885	3202 ± 1381
Monocytes ($x10^6$/L)	267 ± 139	312 ±149	284 ± 140	281 ± 132	231 ± 159
Eosinophils ($x10^6$/L)	145 ± 162	103 ± 99	155 ± 143	134 ± 159	83 ± 68
Basophils ($x10^6$/L)	9.3 ± 19.3	6.9 ± 14.1	8.5 ± 14.6	6.6 ± 11.8	8.1 ± 15.8
Bands	32.6 ± 44.1	35.1 ± 54.8	25.2 ± 25.9	19.7 ± 22.5	40.1 ± 45.0
Atypical lymphocytes	.10 ± .22	.23 ± .40	.16 ± .31	.24 ± .43	.02 ± .08
Ct breaks, deletions, exchanges [D]	1.20 ± 1.36	1.55 ± 1.30	1.80 ± 1.35	2.50 ± 1.61	2.18 ± 1.70

Variable [E]	Unexposed	.1 - <30 ppm-y	30 - <50 ppm-y	50 - <100 ppm-y	≥100 ppm-y
Total Cs aberrations (except gaps) [B]	.59 ± 1.25	.48 ± .83	1.11 ± 1.08	1.20 ± 1.40	1.00 ± 1.17
Total aberrations (except gaps) [C]	1.78 ± 2.05	2.03 ± 1.61	2.91 ± 1.79	3.70 ± 2.41	3.18 ± 2.31
Chromatid gaps	1.4 ± 1.7	.5 ± 1.1	.5 ± .7	.7 ± 1.4	1.7 ± 2.1
Chromatid breaks [C]	1.2 ± 1.4	1.5 ± 1.3	1.8 ± 1.3	2.5 ± 1.6	2.2 ± 1.6
Chromosome breaks [A]	.20 ± .49	.03 ± .19	.34 ± .64	.43 ± .63	.33 ± .65
Acentrics [A]	.39 ± .98	.38 ± .73	.77 ± .88	.73 ± 1.1	.58 ± .79
Hypodiploidy - 45 chrom.	.22 ± .54	.07 ± .26	.14 ± .36	.23 ± .63	.45 ± .79
Hypodiploidy - ≤ 44 chrom. [B]	2.7 ± 2.7	6.4 ± 6.8	6.3 ± 7.1	8.2 ± 7.7	5.6 ± 8.0
Hyperdiploidy	.06 ± .24	.10 ± .31	.11 ± .40	.23 ± .63	.09 ± .29
Aneuploidy (45 or 47 chrom.) [A]	.27 ± .57	.17 ± .38	.26 ± .61	.47 ± .82	.55 ± .90
Total aneuploidy (≤45, 47 chrom.) [C]	3.0 ± 2.7	6.6 ± 6.8	6.6 ± 7.4	8.7 ± 7.5	6.1 ± 8.0
Stimulated cells/1000 cells	568 ± 126	528 ± 160	603 ± 110	605 ± 124	581 ± 124
Metaphases/1000 cells [A]	45 ± 26	47 ± 24	48 ± 25	55 ± 30	54 ± 22
Benzene oxide albumin adducts (nmol/g) [C]	.18 ± .08	.36 ± .17	.30 ± .14	.52 ± .37	1.06 ± 1.28
1,4-Benzoquinone albumin adducts (nmol/g) [C]	1.1 ± .5	2.4 ± 1.2	1.7 ± 1.1	2.9 ± 2.1	4.8 ± 2.4

[A] $p \leq 0.05$, test for exposure-response trend

[B] $p \leq 0.01$, test for exposure-response trend

[C] $p \leq 0.001$, test for exposure-response trend

[D] $p \leq 0.0001$, test for exposure-response trend

[E] The means and standard deviations reported are the raw variables, but for the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

Table 13. Regression Analyses of Blood Counts, Chromosome Aberrations and Albumin Adducts by "Lifetime" **Cumulative Benzene Exposure** Level, Adjusting for Sex, Age, Cotinine and Toluene Levels [E]

Variable	Benzene Regress. Coeff. ± S.E., Unadjusted	Benzene Regress. Coeff. ± S.E., Adjusted for Sex, Age, Cotinine & Toluene
Red blood cells ($x10^{10}$/L)	-.191 ± .053 [C]	-.123 ± .045 [B]
Hematocrit	.0009 ± .009	.017 ± .009
Platelets ($x10^{9}$/L)	.255 ± .126 [A]	.342 ± .148 [A]
White blood cells ($x10^{6}$/L)	-4.70 ± 1.26 [C]	-4.49 ± 1.30 [C]
Lymphocytes ($x10^{6}$/L)	-1.19 ± .67	-1.16 ± .67
Neutrophils ($x10^{6}$/L)	-3.02 ± .96 [B]	-2.83 ± .98 [B]
Monocytes, log($x10^{6}$/L)	-.002 ± .0005 [C]	-.002 ± .0005 [C]
Eosinophils, log($x10^{6}$/L)	-.003 ± .001 [A]	-.003 ± .001 [A]
Basophils, log($x10^{6}$/L)	-.0006 ± .001	-.0008 ± .001
Bands (√)	.003 ± .003	.004 ± .003
Atypical lymphocytes (√)	-.0005 ± .0003	-.0005 ± .0003
Ct breaks, deletions, exchanges (√)	.001 ± .0005 [A]	.001 ± .0005 [A]
Total Cs aberrations (except gaps) (√)	.0009 ± .0005 [A]	.0008 ± .0004 [A]
Total aberrations (except gaps) (√)	.002 ± .0006 [B]	.002 ± .0006 [B]
Chromatid gaps (√)	.001 ± .0005 [B]	-.0003 ± .0006
Chromatid breaks (√)	.001 ± .0005 [B]	.001 ± .0005 [A]
Chromosome breaks (√)	.0005 ± .0002 [A]	.0005 ± .0003
Acentrics (√)	.0004 ± .0003	.0003 ± .0004
Hypodiploidy - 45 chrom. (√)	.0008 ± .0002 [B]	.0008 ± .0002 [C]
Hypodiploidy - ≤ 44 chrom. (√)	.0008 ± .001	.00005 ± .001
Hyperdiploidy - 47 chrom (√)	0 ± .0002	.00003 ± .0002
Aneuploidy (45 or 47 chrom.) (√)	.0008 ± .0003 [B]	.0008 ± .0003 [B]
Total aneuploidy (≤45, 47 chrom.) (√)	.001 ± .001	.0004 ± .001
Stimulated cells/1000 cells	.07 ± .12	.10 ± .12
Metaphases/1000 cells	.031 ± .023	.033 ± .023

Variable	Benzene Regress. Coeff. ± S.E., Unadjusted	Benzene Regress. Coeff. ± S.E., Adjusted for Sex, Age, Cotinine & Toluene
Benzene oxide adducts, (log nmol/g)	.002 ± .0002 [D]	.002 ± .0002 [D]
1,4-Benzoquinone adducts, (log nmol/g)	.003 ± .0004 [D]	.003 ± .0004 [D]

[A] $p \leq 0.05$, test for exposure-response trend
[B] $p \leq 0.01$, test for exposure-response trend
[C] $p \leq 0.001$, test for exposure-response trend
[D] $p \leq 0.0001$, test for exposure-response trend

[E] The controls were included in the analysis and were assigned an exposure value of 0.

Table 14. Means and Standard Deviations (or Percents) for Blood Count, Chromosome Aberration and Ploidy, and Albumin Adduct Variables, Grouped According to **Benzene Exposure Duration** (Years)

Variable [D]	Unexposed	<5 y	5- <10 y	10- <18 y	18+ y
No. of subjects	51	44	38	28	20
Female (%)	53%	39%	50%	68%	65%
Smokers (%; reported + cotinine)	31%	45%	45%	25%	30%
Mean Age	33.3 ± 7.4	32.2 ± 9.5	38.8 ± 5.8	36.5 ± 4.3	40.0 ± 5.6
Mean cumulative benzene exposure (ppm-years)	0	78 ± 86	84 ± 82	77 ± 122	90 ± 55
Mean benzene exposure level per year (ppm)	0	28.2 ± 40.2	13.3 ± 13.0	6.4 ± 10.5	4.6 ± 2.9
Current benzene exposure (mean over 4 weeks) (ppm)	0	14.5 ± 16.7	16.2 ± 34.6	3.1 ± 4.0	5.0 ± 5.9
Red blood cells ($x10^{10}$/L) [c]	463 ± 52	407 ± 59	403 ± 60	400 ± 46	383 ± 57
Hematocrit (year 2 only)	44.2 ± 5.3	43.1 ± 3.4	42.8 ± 4.1	42.6 ± 3.5	43.3 ± 5.3
Platelets ($x10^9$/L; year 2 only)	277 ± 43	284 ± 60	278 ± 51	284 ± 72	275 ± 92
White blood cells ($x10^6$/L)	6671 ± 1502	5955 ± 1226	6279 ± 1543	6146 ± 1436	6010 ± 1676
Lymphocytes ($x10^6$/L)	2205 ± 789	2250 ± 630	2300 ± 707	2311 ± 826	2396 ± 948
Neutrophils ($x10^6$/L) [B]	4006 ± 1108	3304 ± 929	3488 ± 1275	3430 ± 990	3144 ± 986
Monocytes ($x10^6$/L)	267 ± 139	271 ± 149	285 ± 138	266 ± 100	302 ± 205
Eosinophils ($x10^6$/L)	145 ± 162	92 ± 66	164 ± 180	104 ± 113	115 ± 83
Basophils ($x10^6$/L)	9.3 ± 19.3	6.1 ± 12.2	6.3 ± 14.9	8.5 ± 13.4	12.1 ± 17.0
Bands	32.6 ± 44.1	29.5 ± 33.6	28.0 ± 50.8	29.8 ± 36.9	35.9 ± 31.4
Atypical lymphocytes	.10 ± .22	.19 ± .39	.16 ± .34	.14 ± .31	.13 ± .27

Variable [D]	Unexposed	<5 y	5- <10 y	10- <18 y	18+ y
Ct breaks, deletions, exchanges [C]	1.20 ± 1.4	1.74 ± 1.5	1.79 ± 1.3	2.27 ± 1.6	2.65 ± 1.7
Total Cs aberrations (except gaps) [A]	0.59 ± 1.25	.95 ± 1.19	.87 ± 1.12	.96 ± 1.18	1.15 ± 1.18
Total aberrations (except gaps) [C]	1.78 ± 2.05	2.70 ± 2.09	2.66 ± 1.95	3.23 ± 2.05	3.80 ± 2.44
Chromatid gaps [C]	1.4 ± 1.7	1.3 ± 2.0	.61 ± 1.1	.62 ± .75	.60 ± 1.5
Chromatid breaks [C]	1.2 ± 1.4	1.7 ± 1.5	1.8 ± 1.3	2.3 ± 1.6	2.6 ± 1.6
Chromosome breaks	.20 ± .49	.30 ± .64	.29 ± .57	.35 ± .56	.20 ± .52
Acentrics [B]	.39 ± .98	.51 ± .74	.58 ± .86	.62 ± .98	.95 ± 1.1
Hypodiploidy - 45 chrom.	.22 ± .54	.21 ± .56	.32 ± .70	.31 ± .55	0
Hypodiploidy - ≤ 44 chrom. [C]	2.7 ± 2.7	4.5 ± 5.9	9.1 ± 8.9	6.6 ± 7.9	6.7 ± 5.5
Hyperdiploidy	.06 ± .24	.14 ± .35	.11 ± .31	.15 ± .46	.15 ± .67
Aneuploidy (45 or 47 chrom.)	.27 ± .57	.35 ± .72	.42 ± .72	.46 ± .76	.15 ± .67
Total aneuploidy (≤45, 47 chrom.) [C]	3.0 ± 2.7	4.8 ± 5.9	9.5 ± 8.9	7.1 ± 8.0	6.9 ± 5.4
Stimulated cells/1000 cells	568 ± 126	587 ± 151	554 ± 108	591 ± 132	602 ± 131
Metaphases/1000 cells	45 ± 26	59 ± 25	49 ± 28	47 ± 20	44 ± 24
Benzene oxide albumin adducts (nmol/g)	.18 ± .08	.82 ± 1.17	.53 ± .37	.35 ± .26	.40 ± .28
1,4-Benzoquinone albumin adducts (nmol/g)	1.1 ± .5	3.9 ± 2.4	3.2 ± 1.7	1.9 ± 1.7	2.0 ± 1.7

[A] $p \leq 0.05$, test for exposure-response trend
[B] $p \leq 0.01$, test for exposure-response trend
[C] $p \leq 0.001$, test for exposure-response trend
[D] The means and standard deviations reported are the raw variables, but for the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

Table 15. Means and Standard Deviations (or Percents) for Blood Count, Chromosome Aberration and Ploidy, and Albumin Adduct Variables, Grouped According to Average Lifetime Occupational **Benzene Exposure Concentration** (ppm)

Variable [D]	Unexposed	>0 - <5 ppm	5- <15 ppm	15- <40 ppm	40+ ppm
No. of subjects	51	54	36	29	11
Female (%)	53%	54%	53%	48%	55%
Smokers (%; reported + cotinine)	31%	35%	42%	45%	27%
Mean Age	33.3 ± 7.4	35.2 ± 7.6	39.3 ± 5.2	36.3 ± 8.7	31.4 ± 8.6
Mean cumulative benzene exposure (ppm-years)	0	32 ± 21	74 ± 51	123 ± 65	237 ± 188
Mean years of benzene exposure	5.6 ± 8.8	11.2 ± 6.4	9.8 ± 6.0	4.4 ± 1.9	4.0 ± 3.2
Current benzene exposure (mean over 4 weeks) (ppm)	0	3.07 ± 2.9	5.89 ± 4.8	17.4 ± 15.5	50.6 ± 55.4
Red blood cells ($x10^{10}$/L) [C]	463 ± 52	403 ± 62	396 ± 57	404 ± 51	391 ± 39
Hematocrit (year 2 only)	44.2 ± 5.3	43.0 ± 3.7	42.7 ± 4.7	43.1 ± 3.3	--
Platelets ($x10^9$/L; year 2 only)	277 ± 43	271 ± 69	292 ± 72	288 ± 38	--
White blood cells ($x10^6$/L) [C]	6671 ± 1502	6383 ± 1330	6089 ± 1455	6103 ± 1560	4727 ± 548
Lymphocytes ($x10^6$/L)	2205 ± 789	2551 ± 797	2136 ± 779	2169 ± 559	1956 ± 395
Neutrophils ($x10^6$/L) [C]	4006 ± 1108	3377 ± 868	3491 ± 1121	3501 ± 1314	2480 ± 451
Monocytes ($x10^6$/L)	267 ± 139	298 ± 141	280 ± 157	277 ± 145	185 ± 110
Eosinophils ($x10^6$/L) [A]	145 ± 162	118 ± 104	144 ± 131	108 ± 163	71 ± 52
Basophils ($x10^6$/L)	9.3 ± 19.3	7.9 ± 13.6	9.1 ± 16.1	6.8 ± 14.5	3.1 ± 6.9
Bands	32.6 ± 44.1	31.6 ± 44.0	26.5 ± 31.7	31.7 ± 43.5	30.6 ± 30.0
Atypical lymphocytes	.10 ± .22	.20 ± .35	.19 ± .36	.11 ± .33	0
Ct breaks, deletions, exchanges [B]	1.20 ± 1.4	1.89 ± 1.4	2.23 ± 1.6	1.89 ± 1.6	2.18 ± 1.7

Variable [D]	Unexposed	>0 - <5 ppm	5- <15 ppm	15- <40 ppm	40+ ppm
Total Cs aberrations (except gaps) [A]	.59 ± 1.3	.85 ± 1.0	1.00 ± 1.4	1.14 ± 1.1	.91 ± 1.2
Total aberrations (except gaps) [B]	1.78 ± 2.05	2.74 ± 1.95	3.23 ± 2.25	3.03 ± 2.10	3.09 ± 2.63
Chromatid gaps	1.4 ± 1.7	.58 ± 1.2	.37 ± .88	1.3 ± 1.9	2.6 ± 1.7
Chromatid breaks [B]	1.2 ± 1.4	1.9 ± 1.4	2.2 ± 1.6	1.9 ± 1.6	2.2 ± 1.7
Chromosome breaks [A]	.20 ± .49	.15 ± .36	.43 ± .78	.32 ± .55	.45 ± .69
Acentrics	.39 ± .98	.66 ± .92	.57 ± .88	.75 ± .93	.27 ± .65
Hypodiploidy - 45 chrom. [A]	.22 ± .54	.09 ± .30	.14 ± .36	.50 ± .92	.45 ± .69
Hypodiploidy - ≤ 44 chrom. [A]	2.7 ± 2.7	6.7 ± 7.0	7.1 ± 6.7	7.0 ± 9.7	3.4 ± 3.6
Hyperdiploidy	.06 ± .24	.09 ± .35	.23 ± .60	.07 ± .26	.18 ± .40
Aneuploidy (45 or 47 chrom.) [B]	.27 ± .57	.19 ± .52	.37 ± .65	.57 ± 1.03	.64 ± .67
Total aneuploidy (≤45, 47 chrom.) [B]	3.0 ± 2.7	6.9 ± 7.1	7.5 ± 6.7	7.6 ± 9.6	4.0 ± 4.0
Stimulated cells/1000 cells	568 ± 126	573 ± 128	573 ± 148	574 ± 117	657 ± 127
Metaphases/1000 cells [A]	45 ± 26	48 ± 25	49 ± 25	53 ± 24	65 ± 30
Benzene oxide albumin adducts (nmol/g) [C]	.18 ± .08	.32 ± .16	.45 ± .26	.94 ± 1.33	1.17 ± .80
1,4-Benzoquinone albumin adducts (nmol/g) [C]	1.1 ± .5	2.0 ± 1.2	2.5 ± 1.7	4.1 ± 2.4	6.0 ± 2.0

[A] $p \leq 0.05$, test for exposure-response trend

[B] $p \leq 0.01$, test for exposure-response trend

[C] $p \leq 0.001$, test for exposure-response trend

[D] The means and standard deviations reported are the raw variables, but for the monocytes, eosinophils, basophils and albumin adducts the statistical tests were performed on the log transformed data, while for all the chromosome aberration and ploidy variables the means are per 100 cells and the statistical tests were performed on square-root transformed data.

Table 16. Regression Analyses of Blood Counts, Chromosome Aberrations and Albumin Adducts by **Benzene Exposure Duration and Average Concentration** when Both are Included in the Model, Adjusting for Sex, Age, Cotinine and Toluene Levels [B]

Variable	Benzene Duration, Regress. Coeff. ± S.E. (p-value) [A]	Benzene Average Concentration, Regress. Coeff. ± S.E. (p-value) [A]
Red blood cells ($x10^{10}$/L)	-5.4 ± 3.8	-10.1 ± 3.4 (.003)
White blood cells ($x10^{6}$/L)	-165 ± 111	-365 ± 98 (.0003)
Lymphocytes ($x10^{6}$/L)	32 ± 58	-120 ± 51 (.02)
Neutrophils ($x10^{6}$/L)	-182 ± 84 (.03)	-209 ± 74 (.005)
Monocytes, log($x10^{6}$/L)	-.04 ± .04	-.10 ± .04 (.008)
Eosinophils, log($x10^{6}$/L)	-.10 ± .09	-.17 ± .08 (.04)
Ct breaks, deletions, exchanges (√)	.017 ± .06 (.01)	.003 ± .002
Total Cs aberrations (except gaps) (√)	-.0005 ± .005	.003 ± .001
Total aberrations (except gaps) (√)	.014 ± .007 (.05)	.004 ± .002(.03)
Chromatid gaps (√)	.010 ± .006 (.09)	.008 ± .002(.0001)
Chromatid breaks (√)	.017 ± .006 (.01)	.003 ± .002
Chromosome breaks (√)	-.003 ± .003	.0007 ± .001
Acentrics (√)	.070 ± .030 (.02)	.003 ± .009
Hypodiploidy - 45 chrom. (√)	.020 ± .020	.059 ± .018 (.001)
Hypodiploidy - ≤ 44 chrom. (√)	.059 ± .085	-.023 ± .075
Hyperdiploidy - 47 chrom (√)	.011 ± .015	.015 ± .013
Aneuploidy (45 or 47 chrom.) (√)	.030 ± .024	.072 ± .021 (.0008)
Total aneuploidy (≤45, 47 chrom.) (√)	.073 ± .084	.014 ± .074
Stimulated cells/1000 cells	14.9 ± 10.1	16.7 ± 8.9 (.06)
Metaphases/1000 cells	-1.7 ± 2.0	4.3 ± 1.7 (.01)
Benzene oxide adducts, (log nmol/g)	-.016 ± .015	.134 ± .013 (<.0001)
1,4-Benzoquinone adducts, (log nmol/g)	-.050 ± .028 (.08)	.272 ± .025 (<.0001)

[A] P-values ≤0.10 are shown.

[B] Controls were included in the analyses and were assigned zero values for duration and concentration of benzene exposure.

Table 17. **Linear-Quadratic** Model: Shape of the Exposure-Response Relationship for Blood, Adduct and Chromosome-Aberration Variables [A,B]

	Including the Unexposed Group		Excluding the Unexposed Group	
	Linear Term	Quadratic Term	Linear Term	Quadratic Term
Benzene oxide adducts, log(nmol/g)	.03 ± .003 (<.0001)	-.04 ± .007 (<.0001)	.03 ± .004 (<.0001)	-.03 ± .008 (.0001)
1,4-Benzoquinone adducts, log(nmol/g)	.07 ± .006 (<.0001)	-.08 ± .01 (<.0001)	.06 ± .008 (<.0001)	-.07 ± .02 (<.0001)
Red blood cells ($x10^{10}$/L)	-3.3 ± 1.0 (.002)	5.1 ± 2.1 (.02)	-.20 ± 1.1 (--)	.14 ± 2.2 (--)
White blood cells ($x10^{6}$/L)	-37 ± 27 (--)	10 ± 56 (--)	-21 ± 30 (--)	-17 ± 60 (--)
Lymphocytes ($x10^{6}$/L)	7.6 ± 14 (--)	-33 ± 29 (--)	-2.1 ± 16 (--)	-17 ± 31 (--)
Neutrophils ($x10^{6}$/L)	-44 ± 20 (.03)	45 ± 42 (--)	-19 ± 22 (--)	3.4 ± 45 (--)
Ct aberrations (√)	.006 ± .004 (--)	-.003 ± .003 (--)	-.003 ± .012 (--)	-.004 ± .02 (--)
Cs aberrations (√)	.012 ± .009 (--)	-.016 ± .02 (--)	.001 ± .01 (--)	.003 ± .02 (--)
Ct + Cs aberrations (√)	.02 ± .01 (.08)	-.03 ± .03 (--)	.002 ± .01 (--)	.006 ± .03 (--)
Aneuploidy (45, 47 chromosomes) (√)	.004 ± .006 (--)	-.005 ± .01 (--)	.002 ± .007 (--)	-.002 ± .01 (--)
Aneuploidy (<45, 45, 47 chromosomes)(√)	.03 ± .02 (--)	-.06 ± .04 (--)	-.01 ± .03 (--)	.01 ± .01 (--)

[A] P-values are given only if the p-value is ≤ 0.10. Quadratic coefficients were based on $((\text{benzene exposure in ppm})/10)^2$. All analyses controlled for age, sex and cotinine.

[B] The controls were randomly assigned values between 0 and 0.01 ppm which was the approximate lower limit of detection.

Table 18. **Interactions by Sex**: Regression Analyses of Urine Biomarkers by Benzene Exposure Level (Day of Urine Collection) to Examine Interactions with Sex, Adjusting for Dose and Sex Main Effects, and Optionally for Toluene and Cotinine Effects [B]

Variable	Sex Interaction (F/M greater effect); Interaction p-value	Benzene Regression Coeff. ± S.E. for Males	Benzene Regression Coeff. ± S.E. for Females
t,t-MA (log mg/g cr.), pre-work	(F); <.0001	.011 ± .003	.038 ± .004
t,t-MA (log mg/g cr.), post-work	(F); <.0001	.023 ± .006	.056 ± .006
t,t-MA (log mg/g cr.), post – pre	(F); .08	.013 ± .005	.018 ± .005
S-PMA (log µg/g cr.), pre-work	(F); <.0001	.014 ± .011	.077 ± .012
S-PMA (log µg/g cr.), post-work	(F); <.0001	.054 ± .012	.104 ± .012
S-PMA (log µg/g cr.), post – pre	-- [A]		
Phenol (log mg/g cr.), pre-work	(F); .0002	-.002 ±.011	.044 ± .011
Phenol (log mg/g cr.), post-work	(F); .008	.046 ± .011	.083 ± .010
Phenol (mg/g cr.), post – pre	--		
t,t-MA/S-PMA ratio (log)	(M); .08	-.001 ± .001	-.003 ± .001
Red blood cells ($x10^{10}$/L)	--		
Hematocrit	--		
Platelets ($x10^{9}$/L)	--		
White blood cells ($x10^{6}$/L)	--		
Lymphocytes ($x10^{6}$/L)	--		
Neutrophils ($x10^{6}$/L)	--		
Monocytes, log($x10^{6}$/L)	--		
Eosinophils, log($x10^{6}$/L)	--		
Basophils, log($x10^{6}$/L)	--		
Bands (√)	--		
Atypical lymphocytes (√)	--		
Chromatid gaps (√)	--		
Chromatid breaks (√)	--		
Ct breaks, deletions, exchanges (√)	--		
Chromosome breaks (√)	--		
Acentrics (√)	--		

Total Cs aberrations (except gaps) (√)	(F); .03	-.002 ± .004	.010 ± .004
Total aberrations (except gaps) (√)	--		
Hypodiploidy - 45 chrom. (√)	--		
Hypodiploidy - ≤ 44 chrom. (√)	--		
Hyperdiploidy (√)	--		
Aneuploidy (45 or 47 chrom.) (√)	--		
Total aneuploidy (≤45, 47 chrom.) (√)	--		
Stimulated cells/1000 cells	--		
Metaphases/1000 cells	--		
Benzene oxide adducts, log(nmol/g)	--		
1,4-Benzoquinone adducts, log(nmol/g)	(F); .06	.022 ± .003	.029 ± .003

[A] P-values > 0.10 for the interaction terms are not given.

[B] The controls were included in the analysis and were randomly assigned values between 0 and 0.01 ppm which was the approximate lower limit of detection.

Table 19. **Interactions by Smoking Status**: Regression Analyses of Urine, Blood and Chromosome Biomarkers by Benzene Exposure Level to Examine Interactions with Smoking, Adjusting for Dose and Smoking Main Effects, and Optionally for Age and Toluene. Interaction Analyses are also Given for a Low-Exposure Subset (<5 ppm) [C]

Variable	Smoking Interaction (S/NS: Smoker/-Nonsmoker has greater effect); p-value	Low-Exposure (<5 ppm) Smoking Interaction	Benzene Regression Coeff. ± S.E. for Nonsmokers	Benzene Regression Coeff. ± S.E. for Smokers
t,t-MA (log mg/g cr.), pre-work	(NS); .04	-- [A]	.032 ± .003	.009 ± .006
t,t-MA (log mg/g cr.), post-work	-- [A]	(S) ; .08	.046 ± .005	.028 ± .008
t,t-MA (log mg/g cr.), post - pre	--	--		
S-PMA (log µg/g cr.), pre-work	--	--		
S-PMA (log µg/g cr.), post-work	--	--		
S-PMA (log µg/g cr.), post - pre	--	(NS); .08	.032 ± .007	.038 ± .012
Phenol (log mg/g cr.), pre-work	--	--		
Phenol (log mg/g cr.), post-work	--	--		
Phenol (mg/g cr.), post - pre	(NS); .003	--	3.02 ± .32	.68 ± .35
t,t-MA/S-PMA ratio (log)	--	--		
Red blood cells ($x10^{10}$/L)	--	--		
Hematocrit	-- [A]	--		
Platelets ($x10^{9}$/L)	--	--		
White blood cells ($x10^{6}$/L)	--	--		
Lymphocytes ($x10^{6}$/L)	--	--		
Neutrophils ($x10^{6}$/L)	--	--		
Monocytes, log($x10^{6}$/L)	--	--		
Eosinophils, log($x10^{6}$/L)	--	--		
Basophils, log($x10^{6}$/L)	--	--		
Bands (√)	--	--		
Atypical lymphocytes (√)	--	--		
Ct breaks, deletions, exchanges (√)	(S); .10	--	-.002 ± .004	.010 ± .006
Total Cs aberrations (except gaps) (√)	--	--		

Variable	Smoking Interaction (S/NS: Smoker/-Nonsmoker has greater effect); p-value	Low-Exposure (<5 ppm) Smoking Interaction	Benzene Regression Coeff. ± S.E. for Nonsmokers	Benzene Regression Coeff. ± S.E. for Smokers
Total aberrations (except gaps) (√)	(S); .05	--	.0001 ± .005	.011 ± .006
Chromatid gaps (√)	(S); .10	(S); .03	.012 ± .004	.004 ± .006
Chromatid breaks (√)	(S); .06	--	-.002 ± .004	.010 ± .006
Chromosome breaks (√)	--	--		
Acentrics (√)	--	--		
Hypodiploidy - 45 chrom. (√)	(NS); .08	--	.006 ± .002	-.002 ± .003
Hypodiploidy - ≤ 44 chrom. (√)	--	--		
Hyperdiploidy (√)	--	--		
Aneuploidy (45 or 47 chrom.) (√)	--	--		
Total aneuploidy (≤45, 47 chrom.) (√)	--	--		
Stimulated cells/1000 cells	--	--s		
Metaphases/1000 cells	--	--		
Benzene oxide adducts, log(nmol/g)	--	--		
1,4-Benzoquinone adducts, log(nmol/g)	(NS); .05	--	.029 ± .003	.021 ± .004

[A] P-values > 0.10 for the interaction terms are not given.

[B] This comparison of the regression slopes for smokers and nonsmokers is for only those with benzene exposures <5 ppm.

[C] The controls were included in the analysis and were randomly assigned values between 0 and 0.01 ppm which was the approximate lower limit of detection.

Table 20. Means ± Standard Deviations for Urine and Blood Variables, According to **Benzene and Toluene Exposure** Levels [A]

Variable [D]	Unexposed	Low Benzene, Low Toluene	Low Benzene, High Toluene	High Benzene, Low Toluene	High Benzene, High Toluene
No. of subjects	51	45	28	27	30
Female (%)	53%	64%	39%	56%	43%
Smokers (%; reported + cotinine)	31%	29%	46%	33%	50%
Mean Age	33.3 ± 7.4	35.9 ± 6.0	37.6 ± 7.1	32.7 ± 8.9	38.7 ± 8.1
Mean cumulative benzene exposure (ppm-years)	0	46 ± 27	35 ± 23	186 ± 135	82 ± 58
Mean years of benzene exposure	0	10.3 ± 6.0	7.7 ± 5.3	5.0 ± 3.5	10.4 ± 7.3
Current benzene exposure (mean over 4 weeks) (ppm)	.004 ± .003	1.8 ± 1.3	3.0 ± 1.1	28.1 ± 16.6	12.3 ± 10.6
Geometric mean (over 4 weeks) toluene exposure (ppm)	.004 ± .003	13.3 ± 4.8	28.7 ± 12.9	4.4 ± 4.4	46.3 ± 40.8
t,t-MA (mg/g cr.), pre-work	.34 ± .57	.66 ± .67	.95 ± .56	5.3 ± 6.9	2.2 ± 2.0
t,t-MA (mg/g cr.), post-work	.26 ± .27	2.1 ± 2.6	3.3 ± 2.5	14.2 ± 7.3	7.7 ± 5.2
t,t-MA (mg/g cr.), post - pre	-.08 ± .62	1.5 ± 2.6	2.4 ± 2.5	8.8 ± 7.1	5.4 ± 4.1
S-PMA (µg/g cr.), pre-work	1.8 ± 4.3	46 ± 67	40 ± 58	113 ± 152	148 ± 239
S-PMA (µg/g cr.), post-work	1.9 ± 1.9	98 ± 140	84 ± 124	410 ± 384	643 ± 1195
S-PMA (µg/g cr.), post - pre	.07 ± 4.5	51 ± 129	44 ± 113	297 ± 361	495 ± 1063
Phenol (mg/g cr.), pre-work	11.7 ± 21	8.5 ± 23	8.9 ± 14	33 ± 65	13 ± 18
Phenol (mg/g cr.), post-work	6.9 ± 10.0	12.4 ± 15.7	9.5 ± 9.5	90 ± 98	23 ± 26
Phenol (mg/g cr.), post - pre	-4.9 ± 21	3.9 ± 18	.7 ± 17	60 ± 117	10 ± 30
t,t-MA/S-PMA ratio (log)	.23 ± .25	.06 ± .06	.12 ± .14	.07 ± .10	.05 ± .06

Variable [D]	Unexposed	Low Benzene, Low Toluene	Low Benzene, High Toluene	High Benzene, Low Toluene	High Benzene, High Toluene
Red blood cells ($x10^{10}$/L)	463 ± 52	392 ± 58	412 ± 60	400 ± 44	404 ± 61
Hematocrit (year 2 only)	44.2 ± 5.3	42.9 ± 3.5	43.0 ± 3.6	42.5 ± 3.5	43.0 ± 5.1
Platelets ($x10^9$/L; year 2 only)	277 ± 43	276 ± 65	287 ± 57	244 ± 11	284 ± 79
White blood cells ($x10^6$/L)	6671 ± 1502	6671 ± 1502	6364 ± 1311	5211 ± 955	6130 ± 1823
Lymphocytes ($x10^6$/L)	2205 ± 789	2205 ± 789	2422 ± 741	1970 ± 502	2284 ± 852
Neutrophils ($x10^6$/L)	4006 ± 1108	3498 ± 921	3609 ± 993	2928 ± 757	3311 ± 1408
Monocytes ($x10^6$/L)	267 ± 139	298 ± 145	282 ± 144	204 ± 107	315 ± 162
Eosinophils ($x10^6$/L)	145 ± 162	102 ± 91	123 ± 105	72 ± 57	183 ± 190
Basophils ($x10^6$/L)	9.3 ± 19.3	10.7 ± 15.5	3.0 ± 8.0	4.3 ± 9.9	10.2 ± 17.9
Bands	32.6 ± 44.1	34 ± 32	33 ± 52	30 ± 40	23 ± 37
Atypical lymphocytes	.10 ± .22	.17 ± .35	.34 ± .48	.04 ± .14	.09 ± .21
Ct breaks, deletions, exchanges	1.2 ± 1.4	2.2 ± 1.6	1.6 ± 1.4	2.3 ± 1.6	1.8 ± 1.4
Total Cs aberrations (except gaps)	.59 ± 1.3	1.11 ± 1.3	.75 ± 1.0	1.15 ± 1.2	.75 ± 1.1
Total aberrations (except gaps)	1.8 ± 2.1	3.4 ± 2.1	2.4 ± 1.9	3.4 ± 2.2	2.5 ± 2.1
Chromatid gaps	1.4 ± 1.7	0.7 ± 1.1	0.4 ± .8	2.3 ± 2.0	0.3 ± 1.1
Chromatid breaks	1.2 ± 1.4	2.2 ± 1.5	1.6 ± 1.4	2.3 ± 1.6	1.8 ± 1.4
Chromosome breaks	.20 ± .49	.33 ± .64	.11 ± .32	.42 ± .64	.29 ± .60
Acentrics	.39 ± .98	.73 ± .99	.64 ± .99	.58 ± .81	.46 ± .69
Hypodiploidy - 45 chrom.	.22 ± .54	.16 ± .37	.07 ± .26	.65 ± .85	.11 ± .57
Hypodiploidy - ≤ 44 chrom.	2.7 ± 2.7	6.8 ± 7.7	7.3 ± 6.2	2.6 ± 2.5	9.5 ± 9.6
Hyperdiploidy	.06 ± .24	.20 ± .59	.11 ± .32	.15 ± .37	.04 ± .19

Variable [D]	Unexposed	Low Benzene, Low Toluene	Low Benzene, High Toluene	High Benzene, Low Toluene	High Benzene, High Toluene
Aneuploidy (45 or 47 chrom.)	.27 ± .57	.36 ± .71	.18 ± .39	.81 ± .94	.14 ± .59
Total aneuploidy (≤45, 47 chrom.)	3.0 ± 2.7	7.2 ± 7.8	7.4 ± 6.2	3.4 ± 3.1	9.6 ± 9.5
Stimulated cells/1000 cells	568 ± 126	592 ± 142	564 ± 125	604 ± 113	557 ± 138
Metaphases/1000 cells	45 ± 26	46 ± 23	54 ± 28	64 ± 22	44 ± 25
Benzene oxide albumin adducts (nmol/g)	.18 ± .08	.29 ± .13	.34 ± .13	.97 ± .60	.82 ± 1.3
1,4-Benzoquinone albumin adducts (nmol/g)	1.1 ± .5	1.6 ± 1.4	2.1 ± 0.9	5.2 ± 2.2	3.7 ± 2.0

[A] A high benzene level was defined as >5 ppm and a high toluene level was defined as a geometric mean >20 ppm.

Table 21. Interaction between Benzene and Toluene, and Benzene Regression Coefficients in the **Low-Toluene and High-Toluene Groups** (Benzene-Exposed Group only)

Variable	Toluene Interaction (Low/ High with greater effect); Interaction p-value [A]	Low Toluene Group, Benzene Regression Coeff. ± S.E. [A,B]	High Toluene Grp, Benzene Regress. Coeff. ± S.E. [A,B]
t,t-MA (mg/g cr.), pre-work (log)	--		
t,t-MA (mg/g cr.), post-work (log)	--		
t,t-MA (mg/g cr.), post - pre (log)	--		
S-PMA (µg/g cr.), pre-work (log)	--		
S-PMA (µg/g cr.), post-work (log)	(Hi); .10	.061 ± .010	.104 ± .024
S-PMA (µg/g cr.), post - pre (log)	--		
Phenol (mg/g cr.), pre-work (log)	--		
Phenol (mg/g cr.), post-work (log)	--		
Phenol (mg/g cr.), post - pre	(Lo) ; .03	2.60 ± .42	.677 ± .405
t,t-MA/S-PMA ratio (log)	(Lo); .08	-.0007 ± .0004	-.004 ± .002
Red blood cells ($x10^{10}$/L)	(Lo); .03	.256 ± .356	-1.67 ± .85
Hematocrit (year 2 only)	(Hi); .08	-.53 ± .26	-.054 ± .059
Platelets ($x10^{9}$/L; year 2 only)	--		
White blood cells ($x10^{6}$/L)	--		
Lymphocytes ($x10^{6}$/L)	--		
Neutrophils ($x10^{6}$/L)	--		
Monocytes, log($x10^{6}$/L)	(Hi); .008	-.010 ± .004	.014 ± .007
Eosinophils, log($x10^{6}$/L)	--		
Basophils, log($x10^{6}$/L)	(Hi); .08	-.019 ± .011	.030 ± .022
Bands (√)	--		
Atypical lymphocytes (√)	--		
Ct breaks, deletions, exchanges (√)	(Hi); .03	-.003 ± .004	.006 ± .008
Total Cs aberrations (except gaps) (√)	--		
Total aberrations (except gaps) (√)	--	--	--
Chromatid gaps (√)	Hi; .09	-.007 ± .006	.005 ± .004
Chromatid breaks (√)	--		
Chromosome breaks (√)	--		
Acentrics (√)	--		

Hypodiploidy - 45 chrom. (√)	--		
Hypodiploidy - ≤ 44 chrom. (√)	--		
Hyperdiploidy (√)	--		
Aneuploidy (45 or 47 chrom.) (√)	--		
Total aneuploidy (≤45, 47 chrom.) (√)	--		
Stimulated cells/1000 cells	--		
Metaphases/1000 cells	--		
Benzene oxide adducts, log(nmol/g)	(Hi); .01	.011 ± .001	.020 ± .003
1,4-Benzoquinone adducts, log(nmol/g)	--		

[A] A high toluene level was defined as a geometric mean >20 ppm; the rest were included in the "low toluene" group.

[B] Regression coefficients and their standard errors are given when the one for the quadratic term is ≤0.10.

Table 22. Regression Analyses of the Benzene Exposure-Response (1) Including an **Indicator Variable for Exposed/Unexposed Group**, Adjusted for Age, Sex, Cotinine and Toluene, and (2) Using **Ranked Data** for All Subjects to Evaluate the Sensitivity of the Data to the Metric Chosen

Variable	Benzene Regress. Coeff. ± S.E., Adjusted & Including Indicator Variable	P-value for Regression Coeff. of Ranked Data, Unadjusted
t,t-MA (mg/g cr.), pre-work (log)	.024 ± .003 [D]	<.0001
t,t-MA (mg/g cr.), post-work (log)	.034 ± .004 [D]	<.0001
t,t-MA (mg/g cr.), post - pre (log)	.010 ± .004 [B]	<.0001
S-PMA (µg/g cr.), pre-work (log)	.040 ± .009 [A]	<.0001
S-PMA (µg/g cr.), post-work (log)	.068 ± .008 [D]	<.0001
S-PMA (µg/g cr.), post - pre (log)	.028 ± .006 [D]	<.0001
Phenol (mg/g cr.), pre-work (log)	.021 ± .009 [A]	.02
Phenol (mg/g cr.), post-work (log)	.058 ± .008 [D]	<.0001
Phenol (mg/g cr.), post – pre	2.35 ± .28 [D]	<.0001
Red blood cells ($x10^{10}$/L)	-.24 ± .33	<.0001
White blood cells ($x10^{6}$/L)	-23.9 ± 9.5 [A]	.002
Lymphocytes ($x10^{6}$/L)	-8.8 ± 5.0	.98
Neutrophils ($x10^{6}$/L)	-14.7 ± 7.2 [A]	<.0001
Ct breaks, deletions, exchanges (√)	-.002 ± .004	.04
Total Cs aberrations (except gaps) (√)	.002 ± .003	.07
Total aberrations (except gaps) (√)	-.002 ± .004	.005
Aneuploidy (45 or 47 chrom.) (√)	-.0001 ± .002	.23
Total aneuploidy (≤45, 47 chrom.) (√)	-.002 ± .007	.002
Benzene oxide albumin adducts (nmol/g)	.013 ± .001 [D]	<.0001
1,4-Benzoquinone albumin adducts (nmol/g)	.024 ± .002 [D]	<.0001

[A] $p \leq 0.05$, test for exposure-response trend
[B] $p \leq 0.01$, test for exposure-response trend
[C] $p \leq 0.001$, test for exposure-response trend
[D] $p \leq 0.0001$, test for exposure-response trend

Table 23. Spearman Rank Order Correlations among Selected Biomarkers in the Exposed Group

	ttma, b	ttma, a	ttma, a-b	spma, b	spma, a	spma, a-b	phenol, b	phenol, a	phenol, a-b	rbc	wbc	neutrophils	# metaphases	BO-alb	BQ-alb	tid aberr	chro aberr	tot aberr	aneuploidy
ttma – b	1.0																		
ttma, a	.76	1.0																	
ttma, a-b	.31	.80	1.0																
spma, b	.76	.72	.41	1.0															
spma, a	.67	.85	.64	.85	1.0														
spma, a-b	.08	.48	.63	.07	.55	1.0													
phenol, b	.28	.25	.07	.22	.17	-.07	1.0												
phenol, a	.45	.41	.19	.42	.47	.23	.39	1.0											
phenol, a-b	.27	.28	.16	.29	.38	.30	-.29	.66	1.0										
rbc	-.26	-.26	-.16	-.24	-.31	-.20	.04	-.12	-.15	1.0									
wbc	-.17	-.14	-.04	-.12	-.17	-.11	-.09	-.19	-.15	.23	1.0								
neutrophils	-.25	-.23	-.11	-.20	-.22	-.07	-.11	-.19	-.11	.28	.80	1.0							
# metaphases	.25	.26	.16	.14	.13	.01	.26	.19	-.02	-.03	-.04	-.18	1.0						
BO-alb	.72	.75	.50	.68	.75	.37	.21	.48	.35	-.28	-.23	-.25	.16	1.0					
BQ-alb	.69	.75	.50	.67	.69	.28	.34	.46	.24	-.17	-.19	-.27	.21	.74	1.0				
tid aberr	.08	.13	.14	.13	.18	.16	.02	-.03	-.07	-.23	-.18	-.15	.05	.23	.10	1.0			
chro aberr	.14	.14	.07	.10	.08	-.01	.08	.09	.06	-.11	-.17	-.11	.20	.13	.12	.23	1.0		

	ttma, b	ttma, a	ttma, a-b	spma, b	spma, a	spma, a-b	phenol, b	phenol, a	phenol, a-b	rbc	wbc	neutrophils	# metaphases	BO-alb	BQ-alb	tid aberr	chro aberr	tot aberr	aneuploidy
tot aberr	.09	.15	.13	.12	.16	.13	.06	.01	-.03	-.23	-.22	-.14	.13	.24	.12	.05	.87	1.0	
aneuploidy	.08	.08	.07	.00	.05	.11	.05	.15	.17	-.08	-.08	-.02	.15	.12	.07	.18	.15	-.08	1.0

A correlation of approximately 0.13 to 0.15 is statistically significant, depending on the amount of missing data.

Variable abbreviations: the notations b, a and a-b after the urine biomarkers indicate measurements made before work, after work, or after minus before respectively. Ttma = t,t-MA, spma = S-PMA, rbc = red blood cells, wbc = white blood cells, BO-alb = benzene oxide albumin adducts, BQ-alb = 1,4-benzoquinone albumin adducts, tidaberr = chromatid aberrations, chroaberr = chromosome aberrations (except gaps), tot aberr = total chromosome aberrations (except gaps), and tot aneuploidy = total aneuploidy (≤44, 45 or 47 chromosomes).

Table 24. Spearman Rank-order Correlations of Chromosome Aberrations with **Indicators of High/Low Response to Bone Marrow Toxicants** (i.e., residuals for blood counts and toxicity-related metabolites after controlling for benzene exposure level) in the Exposed Group [A]

Aberrations	T,t-MA [A]	Benzene oxide adducts	1,4-Benzo-quinone adducts	Red Blood Cells	White Blood Cells	No. Neutrophils	No. Lym-phocytes
Chromatid gaps	-.01	.12	.05	.00	-12	-.03	-.01
Chromatid breaks	-.06	.01	-.04	-.09	-.13	.00	-.09
Chromosome gaps	.22 $^{.007}$	.13	.16 $^{.04}$	-.03	-.06	-.06	-.04
Chromosome breaks	.06	.09	.09	.02	-.17 $^{.04}$	-.13	-.11
Acentric fragments	.02	-.02	.03	-.10	-.04	-.04	.02
Total aberrations (other than gaps)	-.02	.04	-.01	-.11	-.17 $^{.03}$	-.05	-.09
Hypodiploidy	.08	.11	.06	-.12	-.07	.05	-.09
Hyperdiploidy	-.15	-.04	-.07	-.06	-.03	-.02	-.04
All aneuploidy	-.02	.07	-.02	-.02	-.09	.03	-.10

[A] Benzene exposure averaged over 4 weeks was regressed on adducts or blood count variables, or exposure for the current day was regressed on t,t-MA, and the studentized residuals were calculated for each observation. These residuals indicate whether they showed greater/less than the expected response to the benzene exposure. These indices of responsiveness were then correlated with the chromosome aberration outcomes.

[B] One-tailed p-values are given when the correlation was in the expected direction and was statistically significant.