# RED BLOOD CELL GLYCEROL LYSIS AND HEMATOLOGIC EFFECTS IN OCCUPATIONAL BENZENE EXPOSURE

**ANA BOGADI-ŠARE,* RAJKA TURK; VIŠNJA KARAČIĆ*, MARIJA ZAVALIĆ,* AND KARMEN TRUTIN-OSTOVIĆ†**

'Institute for Medical Research and Occupational Health
Zagreb, Croatia

†Clinical Hospital Dubrava
Zagreb, Croatia

*Fort?-nine female workers in the shoemaking industry, exposed to a solvent mixture containing benzene and twenty-seven non-exposed controls, were investigated. Concentrations of benzene and toluene in the working atmosphere, as well as benzene and toluene in blood and phenols in pre- and post-shift urine as parameters of biological monitoring, were determined. In order to assess hematotoxic risk, a complete blood cell count with differential, hemoglobin. hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes, serum iron, alkaline phosphatase in neutrophils and red blood cell glycerol lysis time were determined in all subjects.*

*Benzene concentrations in the workplace atmosphere at the shoemaking factory ranged from 1.9 to 14.8 ppm (median=5.9). Significant difference in benzene in blood ($p = 0.005$) and phenol in post-shift urine ($p = 0.003$) between exposed workers and controls confirmed exposure to benzene. Hemoglobin level ($p = 0.02$) and mean corpuscular hemoglobin concentration ($p = 0.0002$) in the shoe workers were lower, and band neutrophils ($p = 0.005$) and mean corpuscular volume ($p = 0.03$) higher; than in controls. Red blood cell glycerol lysis time was significantly higher ($p = 0.000001$) in shoe workers ($X \pm SD = 41.6 \pm 8.9$) than in controls ($X \pm SD = 31.1 \pm 6.5$) and showed a significant correlation with exposure biomarkers.*

1. Address all correspondence to: Ana Bogadi-Sare. M.D., M.Sc., Department of Occupational Health. Institute for Medical Research and Occupational Health, 2 Ksaverska St., P.O. Box 291, 10001 Zagreb, Croatia. Tel.: +3851 22 36 25. Fax: +385 1 22 12 52.

2. Abbreviations: $GLT_{50}$, red blood cell glycerol lysis time; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV. mean corpuscular volume; NIOSH. National Institute for Occupational Safety and Health: WHO. World Health Organization.

3. Key words: benzene, hematologic effects, occupational exposure. red blood cell glycerol lysis time.

**Toxicology and Industrial Health. Vol. 13, No. 4, pp. 485–494**

**ISSN: 0748-2337**

***The results confirm that benzene exposure below 15 ppm may produce qualitative abnormalities. particularly macroerythrocytosis and increased red cell glycerol resistance. in the absence of an overt quantitative decrease in circulating blood cells. Increased resistance to the hemolytic action of glycerol is a potentially useful biological monitoring procedure in medical surveillance of benzene exposed workers. The results of this study suggest that potential threshold concentration for hematologic effects of benzene is lower than 15 ppm.***

## INTRODUCTION

Benzene, a common chemical used in industry and an ubiquitous environmental contaminant, has long been associated with hematopoietic disorders, ranging from mild peripheral blood cytopenias to fatal aplastic anemia and leukemia. Most of these disorders, however, developed in individuals exposed to relatively high benzene concentrations (> 100 ppm) (Aksoy et al., 1971; Aksoy et al., 1972). It is assumed that benzene exposure below 25 ppm (Yardley-Jones et al., 1991) does not have such adverse health effects. Continuous reduction of the permissible exposure limit in the working atmosphere has resulted in decreased benzene emissions and consequently a significant drop in the concentration of ambient benzene. Thus, the question remains whether low-level exposure increases the risk of incurring hematotoxic non-leukemogenic and clinically inapparent effects. It is. therefore, necessary to investigate the appropriate tests capable of detecting early benzene toxicity and suitable for medical surveillance program.

Circulating blood cell count is not a sufficiently sensitive indicator for detection of early benzene hematotoxicity (Goldstein, 1986). In addition to a quantitative decrease in all blood cell types, there is evidence that benzene also leads to qualitative abnormalities of these cells. Such changes may precede an overt decrease in cell counts and therefore be useful for the detection of early benzene toxicity. An increased red cell mean corpuscular volume (Aksoy et al.. 1972: Goldstein. 1983; Goldstein. 1986: Beving et al., 1991), altered red cell osmotic fragility (Aksoy et al., 1972) and impaired activity of alkaline phosphatase in neutrophils (Songnian et al., 1982) were found in persons exposed to benzene.

Red blood cell glycerol lysis time ($GLT_{50}$) is a rapid. simple procedure for estimating the fragility of erythrocytes suspended in glycerol and is useful as a screening test for a number of erythrocyte abnormalities (Gottfried and Robertson, 1974: Posteraro and Gottfried, 1978: Zanella et al., 1983). Goldstein and coworkers (Goldstein, 1988) demonstrated increased resistance of red cells to lysis by glycerol in benzene treated mice, and proposed this method as a screening test for benzene hematotoxicity. In this study, red cell glycerol resistance and other hematological quantitative and qualitative parameters were determined in occupationally exposed individuals in order to detect possible hematologic damage in benzene exposure below 15 ppm and to establish appropriate tests and methods for screening and routine medical surveillance.

The study was carried out in 49 female workers. ages 23–53 years (mean **38).** who were employed in a shoe manufacturing factory, and were exposed to solvent mixture for periods of from 1 to 33 years (mean 17). A group of 27 healthy women, ages 29–46 years (mean 38). who were employed in the confectionery industry, constituted the control group. The shoemaking workers were exposed to benzene as a contaminant in glues. cleaners and paints. The control group was not exposed to solvents, including benzene or any other hematotoxic agent. The groups did not differ with regard to age, lifestyle. smoking habits or alcohol consumption. None of the examined subjects had a history of disease of the hematopoietic system.

Benzene and toluene concentrations in the working atmosphere were measured continuously during the work shift in the middle of the working week. Samples of ***air*** were collected at 10 stationary sampling locations using tubes with Casella sampling pumps (Casella. London, United Kingdom) situated on the working tables at nose height. The sampling flow was 100 $mL.m^{-1}$. The absorbed benzene and toluene on charcoal were desorbed with carbon disulfide and analyzed by gas chromatography with a flame ionization detector **(NIOSH,** 1974). Benzene exposure for each employee was ascertained according to the positions of each workplace within a radius of 3 meters around the sampling pump. Exposure assessment was performed blinded with respect to hematologic measurements.

To examine internal exposure, benzene and toluene in blood and phenol concentration in pre- and post-shift urine were determined. Blood samples were taken before the work shift in the middle of the working week and urine samples were taken before and at the end of the same working day. Benzene and toluene concentrations in blood were determined using head-space gas chromatography (Angerer et al., 1973) and phenol in urine using the gas chromatographic method (Sherwood and Carter, 1970).

Blood samples obtained for determination of complete blood cell count with differential, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocytes, serum iron, alkaline phosphatase in neutrophils and $GLT_{50}$ were collected at the same time **as** for benzene determination and the measurements were performed within three hours of collection.

$GLT_{50}$ is a measure of red cell fragility based on the time necessary for hemolysis of erythrocytes in a mixture of glycerol and buffered saline solution. The test **was** carried out according to the procedure described by Gottfried and Robertson (Gottfried and Robertson. 1974). Briefly, 20 µL of whole blood anticoagulated with heparin was added to 5.0 mL of isotonic buffered saline (9 vol of 0.15 M sodium chloride plus 1 vol of 0.10 M phosphate buffer), pH 7.4. The suspension was carefully mixed and 1 mL transferred into a standard cuvette of an spectrophotometer equipped with a linear/logarithmic potentiometer recorder (Perkin Elmear spectrophotometer and recorder, type 551, Überingen, Germany). With the wavelength fixed at 625 nm and the recorder operating, 2.0 mL of 0.3 M glycerol was quickly added. Using a completely hemolyzed blood sample as a blank, the rate of hemolysis was followed by measuring the fall in turbidity of the reaction mixture. Results were recorded as $GLT_{50}$ values, i.e. the time taken in seconds for initial optical density to fall by half.

Finally, statistical analyses were performed using Student t-test and Wilcoxon rank sum test, in order to determine significant differences in hematologic parameters between the exposed and control group. Spearman's correlation was applied to detect the relation between hematologic values and exposure indicators. The independent variables were age, smoking habits, alcohol consumption, benzene concentrations in the working atmosphere and in blood and urinary phenol before and after the work shift. Hematologic parameters were dependent variables. Statistical analyses were carried out using CSS:STATISTICA, Stat-Soft, Inc. 1993 (Computer program manual, Tulsa, Oklahoma).

## RESULTS

Results of general air sampling in 10 production areas in a shoemaking factory showed that benzene concentration ranged from 1.9 to 14.8 ppm (median 5.9) and toluene concentration from 11.4 to 49.9 ppm (median 24.9) (Table 1). Significant differences in benzene and toluene concentrations in blood and phenol concentration in urine after the work shift between the exposed and control group are shown in Table 2. The concentration of phenol before the work shift did not differ significantly between the groups.

**TABLE 1. Benzene and Toluene Concentrations in the Atmosphere at Different Workplaces of a Shoemaking Factory**

| Production areas | Benzene (pprn) | Toulene (ppm) |
|---|---|---|
| Cutting | 1.9 | 16.3 |
| Cutting and glueing | 3.5 | 25.1 |
| Glueing I | 6.9 | 28.2 |
| Glueing II | 6.1 | 25.4 |
| Fitting | 5.6 | 25.1 |
| Bottoming | 14.6 | 49.9 |
| Finishing | 3.0 | 11.4 |
| Heating and drying | 4.3 | 17.4 |
| Cleaning and inspecting | 5.7 | 29.7 |
| Packing | | |

**TABLE 2. Benzene and Toluene Concentrations in Blood and Urinary Phenol Before and After the Work-Shift in Shoemaking Workers and Control**

| Parameters | Shoe Workers | Controls | $p$ . |
|---|---|---|---|
| Benzene in blood (mg/L) | 0.005 (0.002-0.030) | 0.000 (0.000-0.047) | 0.005 |
| Toulene in blood (mg/L) | o0.099 (0.009–0.879) | 0.000 (0.000-0.009) | 0.000001 |
| Phenol in pre-shift urine (mg/g creatinine) | 3.840 (1.660–100.660) | 2.197 (0.504–9.770) | n.s. |
| Phenol in post-shift unne (mg/g creatinine) | 5.240 (2.150–27.310) | 2.759 (0.540–6.960) | 0.003 |

**Notes:** Results are presented as median (range); $p$, level of significance (Wilcoxon test); and n.s., not significant.

Hematologic parameters are presented in Table 3. Hemoglobin level ($p = 0.02$) and MCHC ($p = 0.0002$) were lower in shoe workers than in controls, while band neutrophils ($p = 0.005$) and MCV ($p = 0.03$) were higher. Red blood cell glycerol lysis time was significantly higher ($p = 0.000001$) in shoe workers ($X \pm SD = 41.6 \pm 8.9$) than in controls ($X \pm SD = 31.2 \pm 6.5$). It is assumed that the same mechanism by which glycerol produces hemolysis is also responsible for macrocytosis (Goldstein et al.. 1988). Therefore, correlation between $GLT_{50}$ and MCV was analyzed. Iron-deficiency anemia is associated with higher $GLT_{50}$ values and normo- and microcytosis and for this reason, microcytosis in persons with iron-deficiency anemia could nullify statistically significant correlation between $GLT_{50}$ and MCV. Hence. correlation was examined after the 5 subjects with low level of serum iron were excluded and positive correlation was found between $GLT_{50}$ and MCV ($r = 0.2521$, $p = 0.0448$).

**TABLE 3. Hematologic Parameters in Shoemaking Workers and Controls**

| Parameter | Shoe workers | Controls | *p* |
|---|---|---|---|
| Erythrocytes ($10^{12}$/L) | 4.127 ± 0.266 | 4.278 ± 0.306 | n.s. |
| Reticulocytes (/$10^3$ E) | 10.5 ± 4.2 | 10.6 ± 2.6 | n.s. |
| Hematocrit (vol %) | 40.3 ± 2.6 | 40.2 ± 2.7 | n.s. |
| Hemoglobin concentration (g/L) | 127.2 ± 12.3 | 133.3 ± 9.0 | 0.02 |
| Mean corpuscular volume (fL) | 98.1 ± 5.4 | 95.3 ± 5.2 | 0.03 |
| Mean corpuscular hemoglobin (pg) | 31.1 ± 2.0 | 31.5 ± 1.8 | n.s. |
| Mean corpuscular hemoglobin concentration (g/L) | 317.7 ± 15.6 | 331.6 ± 12.6 | 0.0002 |
| Serum iron (mmol/L) | 21.4 ± 7.5 | 20.6 ± 6.8 | n.s. |
| Red blood cell glycerol lysis time (sec.) | 41.6 ± 8.9 | 31.2 ± 6.5 | 0.000001 |
| Platelets ($10^9$/L) | 178.31 ± 30.77 | 183.25 ± 39.32 | n.s. |
| Leucocytes ($10^9$/L) | 5.719 ± 1.147 | 6.160 ± 1.680 | n.s. |
| Segmented neutrophils ($10^9$/L) | 3.176 ± 0.799 | 3.538 ± 1.440 | n.s. |
| Band neutrophils ($10^9$/L) | 0.039 ± 0.058 | 0.006 ± 0.018 | 0.005 |
| Eosinophiles ($10^9$/L) | 0.137f0.115 | 0.176 ± 0.297 | n.s. |
| Basophiles ($10^9$/L) | 0.017 ± 0.027 | 0.018 ± 0.035 | n.s. |
| Lymphocytes ($10^9$/L) | 1.896 ± 0.512 | 2.016 ± 0.428 | n.s. |
| Monocytes ($10^9$/L) | 0.381 ± 0.135 | 0.405 ± 0.192 | n.s. |
| Alkaline phosphatase in neutrophils | 59.9 ± 38.3 | 76.9 ± 30.1 | n.s. |

Results are presented as mean and SD; *p*, level of significance (Student r-test); and n.s., not significant.

Table 4 presents correlation coefficients for some hematologic parameters indicative of benzene induced health effects (Aksoy et al., 1971; Aksoy et al., 1972; Goldstein, 1986). In our study, age. smoking habit. and alcohol consumption had no impact on hematological values. Red blood cell count, reticulocytes, hematocrit, hemoglobin, MCV, MCH. serum iron, eosinophile. basophile and monocyte count were not dependent on exposure indicators. $GLT_{50}$ correlated with benzene exposure time, benzene in blood and phenol in post-shift urine as significant benzene exposure biomarkers.

**TABLE 4. Rank Correlation Coefficients for Hematologic Parameters**

| | Benzene exposure time | Benzene[a] | Benzene[b] | Phenol[c] | Phenol[d] |
|---|---|---|---|---|---|
| $GLT_{50}$ | -0.3242[e] | 0.2333 | 0.3642[f] | 0.1767 | 0.4010[f] |
| Platelets | -0.1591 | -0.3799[e] | -0.0601 | -0.0595 | -0.0096 |
| Leucocytes | 0.2042 | 0.1440 | -0.3743[e] | 0.1718 | -0.0248 |
| Segmented neutrophils | -0.0542 | 0.0606 | -0.3976[e] | 0.1853 | 0.0464 |
| Band neutrophils | 0.0077 | 0.4044[f] | 0.2672[e] | 0.1463 | 0.2813[e] |
| Lymphocytes | 0.0574 | 0.0506 | -0.2406[e] | -0.0092 | -0.1722 |

[a]In the working atmosphere.
[b]In blood.
[c]Before the work shift.
[d]After the work shift.
[e]$p<0.05$.
[f]$p<0.01$.

## DISCUSSION

Most benzene-related hematologic effects have been reported in humans occupationally exposed to relatively high concentrations (Aksoy et al., 1971; Aksoy et al, 1972). while the capacity of benzene to cause hematopoietic damage in low concentrations remains controversial. Data are lacking concerning a potential threshold concentration below which adverse effects do not occur in exposed individuals (Mehlman, 1991). Hence. adequate health monitoring of early adverse effects have been questioned (Goldstein, 1984; Goldstein, 1986; Ward et al., 1996).

Some authors investigated occupational exposure to benzene concentrations below 25 ppm by means of a medical surveillance program and found no abnormalities of either quantitative or qualitative hematologic outcomes (Fishbeck et al., 1978; Tsai et al., 1983; Hancock et al., 1984; Kipen et al., 1989; Collins et al.. 1991). Other authors demonstrated that exposure to similar benzene concentrations may result in hematologic effects (Townsend et al., 1978; Yardley-Jones et al., 1988; Rothman et al., 1996; Ward et al., 1996) (Table 5). The present study illustrated altered hematologic response in workers exposed to benzene concentrations below 15 ppm. Lowered hemoglobin level and MCHC confirm that benzene or its metabolites interfere with erythropoiesis, which corroborates the results of previous studies (Aksoy et al., 1971; Aksoy et al., 1972: Guy et al.. 1991). Macroerythrocytosis in shoe workers and a significant difference in MCV between the exposed and non-exposed group also support results of previous studies which emphasized MCV as an early indicator of benzene hematotoxicity (Fishbeck et al., 1978; Goldstein. 1983; Goldstein. 1984; Goldstein, 1986; Kipen et al., 1989; Beving et al., 1991). The results of our study confirm MCV and hemoglobin as hematologic indicators of benzene hematotoxicity in concentrations below 15 ppm.

**TABLE 5. Medical Surveillance Hematologic Parameters in Workers Exposed to Benzene (Modified According to Collins JJ. et al., 1991.)**

| Reference | Benzene exposure (ppm) | Hematologic parameters | | | | |
|---|---|---|---|---|---|---|
| | | RBC | WBC | Pl | Hb | MCV |
| Townsend (1978) | < 2–30 | - | 0 | ? | 0 | 0 |
| Fishbeck (1978) | >25 | 0 | 0 | 0 | - | + |
| | 10 | 0 | 0 | 0 | 0 | 0 |
| Tsai (1983) | co.1–25 | 0 | 0 | 0 | 0 | ? |
| Hancock (1984) | 1.9–3 1.5 | 0 | 0 | ? | 0 | ? |
| Yardely-Jones (1988) | 1–10 | 0 | 0 | 0 | 0 | ? |
| Kipen (1989) | 75 | - | | ? | - | ? |
| | 15–20 | 0 | 0 | ? | 0 | ? |
| Collins (1991) | 0.001–1.40 | 0 | 0 | 0 | 0 | 0 |
| Rothman (1996) | > 31 | - | | | ? | |
| | c 31 | - | 0 | | ? | 0 |
| Bogadi-Šare | < 15 | 0 | 0 | 0 | - | + |

Notes: RBC =red blood cell count; WBC, white blood cell count; Pl, platelet count; Hb, hemoglobin level; MCV, mean corpuscular volume; - , benzene-exposed group is lower than the control group; + , higher in the exposed group; 0, no difference between exposed and controls; ? , data not reported or tests not done.

In the present study we detected the prolongation of red cell glycerol lysis time in workers with low-level benzene exposure. The exact mechanism by which glycerol produces hemolysis is not known, although Goldstein speculated that whatever is responsible for macrocytosis also prolongs the glycerol lysis time (Goldstein et al., 1988). Higher $GLT_{50}$ values were also associated with sickle-cell anemia, thalassemia or other hemoglobinopathies, chronic renal and hepatic disorders and iron-deficiency anemia, that were also characterized by micro and normoerythrocytosis (Posteraro and Gottfried, 1978). Therefore, it is assumed that higher $GLT_{50}$ and increased MCV can distinguish benzene induced hematotoxicity from other hematologic diseases causing prolongation of $GLT_{50}$. However, in our investigation a statistically significant correlation between MCV and $GLT_{50}$ was found after subjects with low serum iron were excluded, i.e. prolonged $GLT_{50}$ was accompanied by increased MCV when the influence of iron-deficiency anemia has been nullified. This result confirms that $GLT_{50}$ in combination with MCV could differentiate benzene induced hematotoxicity from other hematologic diseases generating prolonged $GLT_{50}$.

In some investigations no correlation between hematologic parameters and exposure intensity were found (Townsend et al., 1978; Hancock et al., 1984; Collins et al., 1991). Other studies demonstrated a dose-responserelationship between various measures of current benzene exposure and some hematologic parameters, i.e. leucocytes, lymphocytes and MCV (Rothman et al., 1996)

or white and red blood cell count (Ward et al.. 1996). Some of the hematologic parameters in our study were correlated with exposure indicators. In comparison with hematologic parameters indicative of benzene induced health effects, $GLT_{50}$ showed the most significant correlation with a large number of exposure biomarkers. Our results support Goldstein's opinion that $GLT_{50}$ could be a valuable biomarker of benzene effect in biological monitoring procedure of benzene exposed humans.

The differences in hematologic parameters between workers exposed to benzene concentrations below 15 ppm and controls suggest that the threshold for benzene hematotoxic effects is lower than was assumed. This suggests that even exposure to relatively low levels of benzene (below 15 ppm) may result in a hematologic effect.

Although glues, solvents and paints used are mixtures of various components, our investigation was directed to benzene, in view of the fact that benzene has been described as a hematotoxin (Aksoy et al., 1971; Aksoy et al., 1972; Goldstein, 1977). Although, the ambient toluene concentrations in this study were several times higher than benzene levels, previous findings determined hematotoxic effects of toluene exposure only in cases when the purity of toluene had not been established (Von Burg, 1993; WHO 1993). Toluene is also known as an agent which inhibits benzene metabolism and decreases benzene hematotoxocity (Inoue et al., 1988; Purcell et al., 1990). Thus, the hematologic effects found in our study have been attributed to benzene.

## CONCLUSION

In workers exposed to a benzene concentration below 15 ppm, no cytopenic effects were found, while lower hemoglobin and mean corpuscular hemoglobin concentration and higher mean corpuscular volume and band neutrophils were found. Also, an increase in resistance to the hemolytic action of glycerol. as a possible early sign of benzene hematotoxicity, was observed in accordance with the previous experimental results (Goldstein et al. 1988). In combination with mean corpuscular volume, red blood cell glycerol lysis time could distinguish benzene induced hematotoxicity from other hematologic diseases. Therefore, red blood cell glycerol lysis time could be suitable as a workplace surveillance test. The results of this study suggest that potential threshold concentration for hematologic effects of benzene is below 15 ppm.

## REFERENCES

AKSOY. M., DINCOL. K., AKGUN, T., ERDEM, S, and DINCOL, G. (1971). "Haematological effects of chronic benzene poisoning in 217 workers." Brit. J. Ind. Med. 28:296–302.

AKSOY, M., DINCOL, K., ERDEM. S, AKGÜN, T., and DINCOL, G. (1972). "Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene." Brit. J. Ind. Med. 29:56–64.

ANGERER. J., SZXDOVSKI. D., MANZ. A., PETT, R., and LEHNERT, G. (1973). "Chronische Lösungsmittelbelastung am Arbeitsplatz. I. Gaschromatographische Bestimmung von Benzol und Toluol in der Luft und im Dampfraum von Blutproben." Int. Arch. Arbeitsmed. 31:1–8.

BEVING. H.. TORNLING. G., and OLSSON, P. (1991). "Increased erythrocyte volume in car repair painters and car mechanics." Brit. J. Ind. Med. 48:499–501.

COLLINS. J.J.. CONNER. P.. FRIEDLANDER. B.R.. EASTERDAY. P.A.. NAIR, R.S.. and BRAUN, J. (1991). "A study of the hematologic effects of chronic low–level exposure to benzene." J. Occup. Med. 33:619–26.

FISHBECK, W.A.. TOWNSEND. J.C., and SWANK. M.G. **(1978).** "Effects of chronic occupational exposure to measured concentrations of benzene." J. Occup. Med. 20:539–42.

GOLDSTEIN, B.D. **(1977).** "Hematotoxicity in humans." In: Benzene Toxicity: A Critical Evaluation (S. Laskin and B.D. Goldstein. eds.). J. Toxicol. Environ. Health 1977:2(suppl):69–105.

GOLDSTEIN, B.D. **(1983).** "Clinical hematotoxicity of benzene." In: Carcinogenicity and Toxicity of Benzene (M.A. Mehlman. ed.). Princeton Scientific Publishing Co.. Inc.. Princeton. NJ. pp. 51-61.

GOLDSTEIN. B.D. **(1984).** "Current use of ambient and biological monitoring: reference workplace hazards. organic toxic agents - benzene I." In: Assessment of Toxic Agents at the Workplace (A. Berlin, R.E. Yodaiken. B.A. Henman. eds.). Martinus Nijhoff Publishers. Boston, MA. pp. 161–73.

GOLDSTEIN, B.D. **(1986).** "Biological and ambient monitoring of benzene in the workplace." J. Occup. Med. **28:**1051–4.

GOLDSTEIN, B.D., ROZEN, M.G., and SNYDER. C.A. **(1988).** "Prolonged red blood cell glycerol hemolysis in mice inhaling benzene." Toxicol. Ind. Health 4:499–504.

GOTTFRIED, E.L., and ROBERTSON, N.A. **(1974).** "Glycerol lysis time as a screening test for erythrocyte disorders." J. Lab. Clin. Med. 83:323–33.

GUY. R.L., HU, P.. WITZ, G., GOLDSTEIN, B.D.. and SNYDER. R. **(1991).** "Depression of iron uptake into erythrocytes in mice by treatment with the combined benzene metabolites p–benzoquinone, muconaldehyde and hydroquinone." J. Applied Toxicol. 11:443–6.

HANCOCK, D.G., MOFFITT, A.E.. and HAY. E.B. **(1984).** "Hematological findings among workers exposed to benzene at a coke oven by-product recovery facility." Arch. Environ. Health 39:414–8.

INOUE, O., SEIJI, K., WATANABE, T., KASAHARA, M., NAKATSUKA, H., YIN, S., LI. G.. CAI. S., JIN, C., and IKEDA, M. **(1988).** "Mutual metabolic suppression between benzene and toluene in man." Int. Arch. Occup. Environ. Health **60:**15–20.

KIPEN, H.M., CODY, R.P.. and GOLDSTEIN, B.D. **(1989).** "Use of longitudinal analysis of peripheral blood counts to validate historical reconstructions of benzene exposure." Environ. Health Perspect. 82:**199–206.**

MEHLMAN, M.A. **(1991).** "Benzene health effects: unanswered questions still not addressed." Am. J. Ind. Med. 20:707–711.

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH) **(1974).** HEW Publication No. 74–137, Washington, DC.

POSTERARO, A. and GOTTFRIED, E.L. **(1978).** "The diagnostic significance of a prolonged erythrocytic glycerol lysis time." Am. J. Clin. Pathol. 70:637–41.

PURCELL, K.J., CASON, G.H. and GARGES, M.I. **(1990).** "*In* vivo metabolic interactions of benzene and toluene." Toxicol. Letters 52:141–152.

ROTHMAN, N., GUI-LIN, L.. DOSEMECI. M., BECHTOLD, W.E., MARTI, G.E., WANG, Y., LINET, M., XI, L., LU, W., SMITH, M.T.. TITENKO-HOLAND. N., ZHANG. L.P., BLOT, W., YIN, S.N., and HAYES R.B. **(1996).** "Hematotoxicity among chinese workers heavily exposed to benzene." Am. J. Ind. Med. 29:236–246.

SHERWOOD, R.J., and CARTER, F.W. **(1970).** "The measurement of occupational exposure to benzene vapour." Ann. Occup. Hyp. **13:**125–46.

SONGNIAN, Y. QUILAN, L., and YUXIANG, L. **(1982).** "Significance of leukocyte alkaline phosphatase activity in the diagnosis of chronic benzene poisoning." Reg. Toxicol. Pharmacol. 2:209–12.

TOWNSEND. J.C., OTT. M.G.. and FISHBECK, W.A. **(1978).** "Health exam findings among individuals occupationally exposed to benzene." J. Occup. Med. 20:543–8.

TSAI, S.T.. WEN, C.P., WEISS, N.S., WONG. O., McCLELLAN, W.A., and GIBSON, R.L. **(1983).** "Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries." J. Occup. Med. 25:685–692.

VON BURG, R. (1993). 'Toxicology update: toluene." J. Appi. Toxicol. 13:441–446.

WARD, E., HORNUNG, R., **MORRIS.** J., RINSKY. R., **WILD, D.,** HALPERIN, W., and GUTHRIE, W. (1996). "Risk of low red or white blood cell count related to estimated benzene exposure in a rubberworker cohort (1940-1975)." Am. J. Ind. Med. 29:247–257.

**WORLD HEALTH ORGANUZATION** (WHO) (1993). "Benzene." Environ. Health Criteria 150. Geneva, Switzerland.

YARDLEY-JONES, A., ANDERSON, D.. JENKINSON. **P.C.,** LOVELL. **D.P.,** BLOWERS, **S.D.,** and DAVIES. M.J. (1988). "Genotoxic effects in peripheral blood and urine of workers exposed to low level benzene." Brit. J. Ind. Med. 45:694–700.

YARDLEY–JONES A., ANDERSON, D., and PARKE, D.V. (1991). "'Thetoxicity of benzene and its metabolism and molecular pathology in human risk assessment." Brit. J. Ind. Med. 48:437–44.

ZANELLA, A., MILANI, S., FAGNANI, G., MARIANI, M., SIRCHIA, G. (1983). "Diagnostic value of the glycerol lysis test." J. Lab. Clin. Med. 102:743–50.