The immunotoxicological pattern of subchronic and chronic benzene exposure in rats

Alexander V. Karaulov, Irina V. Mikhaylova, Alexander I. Smolyaugin, Viktor M. Boev, Alexandra Kalogeraki, Aristides M. Tsatsakis, Ayse Basak Engin

A Clinical Immunology and Allergology Department, 2 Bol’shaya Pirogovskaya St., Block 6, I.M. Sechenov First Moscow State Medical University, 119991, Moscow, Russian Federation
B Chemistry and Pharmachemistry Department, Orenburg State Medical University, 6 Sovetskaya St., Orenburg, 460000, Russian Federation
C Fundamental Research Laboratory, Orenburg State Medical University, 45 M.Gorkogo St., Orenburg, 460000, Russian Federation
D Department of Pathology-Cytopathology, Medical School, University of Crete, University Hospital, Heraklion, Crete, Greece
E Department of Forensic Sciences and Toxicology, Medical School, University of Crete, Heraklion, Greece
F Gaz University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey

Abstract

Exposure to benzene and its inevitable metabolites can result in deleterious effects on human health, including lymphopoenia, hematotoxicity and cancer. However, the duration of exposure might alter the effects including immune consequences. The aim of this study was to determine whether benzene could modulate lymphocyte proliferation induced by the T cell mitogen concanavalin A, in rats, at different exposure durations. 386 Wistar rats were assigned into control and treatment groups which were subdivided into groups for 45, 90 and 135 days for 0.6 mL/kg of drinking water mixed benzene treatment. The percentage of CD3+, CD4+, CD8+ spleen lymphocytes was determined using the flow cytometer. Interleukin (IL)-4, IL-6, IL-10 and interferon-gamma, in supernatants of splenocyte cultures stimulated with Concanavalin A, were assessed by enzyme-linked immunosorbent assay (ELISA) technique. The decrease in the total lymphocyte and T cell counts were associated with increased benzene exposure duration. Th2-type cytokine, IL-4 significantly increased, whereas IL-6, CD4 + T cells, CD4+/CD8+ ratio and CD3 + T cells decreased. Despite the positive correlation between benzene toxicity and indicated increased immune responses, 45-day exposure to benzene appeared to be the most sensitive time point for evaluating benzene cytotoxicity.

1. Introduction

Benzene is an aromatic hydrocarbon that is both anthropogenically produced also occurs naturally. In addition to air, it can be found as a contaminant in soil, drinking water and food. Therefore, human can frequently exposed to benzene via several sources, including smoking, exhaust gasses, emissions of industrial, petrochemical and pharmaceutical production processes. Despite its known multiorgan interferences, the sensitive populations, such as children, pregnant women, elderly, immunocompromised individuals and occupational workers are at risk for adverse health effects because of the elevated atmospheric concentrations of benzene (Rich and Orimoloye, 2016). Long term exposure to benzene can cause acute myeloid leukemia (AML) and has effects in the bone marrow, decreasing the red blood cells and other components, that leads to anemia (Agency for Toxic Substances and Disease Registry, 2007). In a recent study by Costa et al. authors assess modifications in circulating levels of advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and serum reactive oxygen metabolites (ROMs) in a group of gasoline station attendants exposed to low-dose benzene and to evaluate the influence of antioxidiant food intake on these biomarkers of oxidative stress and conclude that AOPP are a more sensitive biomarker of oxidative stress in workers exposed to low doses of benzene than AGE (Costa et al., 2016). Chronic exposure to low-dose benzene can modulate signal transduction pathways activated by oxidative stress and involved in cell proliferation and apoptosis. This could represent a possible mechanism of carcinogenic action of chronic benzene exposure (Fenga et al., 2016). Furthermore, it is metabolized into a several intermediate compounds like benzene oxide, catechol, phenol, hydroquinone, and benzoquinones in various organs (Lindstrom et al., 1997). Thus, in addition to benzene, its reactive
metabolites contribute to the alterations in the functions of enzymes and proteins (Uzma et al., 2010). In 1982, International Agency for Research on Cancer (IARC) declared that there is sufficient evidence that benzene is carcinogenic to man and afterwards classified as a Group 1 carcinogen (“Benzene [IARC Summary & Evaluation, Volume 29, 1982],” n.d.). Mincuillo et al. showed that it can either directly damage hematopoietic progenitor cells, leading to the induction of cell death or may alter the responsiveness of these cells to cytokines and cellular adhesion molecules (Mincuillo et al., 2014). Additionally, the duration of the interaction might play a role in immune consequences. Chronic exposure to benzene can lead to deleterious effects on many biological systems including both innate and adaptive components of the immune system, on the other hand, it has been shown that low levels of benzene exposure was associated with significant decline in serum IgM and IgA levels (Kirkeleit et al., 2006). Therefore, we aimed to determine whether benzene could modulate lymphocyte proliferation induced by the T cell mitogen Concanavalin A (Con A), in rats, at different exposure durations.

2. Methods

2.1. Animal experiments

The experiments were conducted with 386 healthy male Wistar rats weighing between 250 and 300 g. Rats were allocated into two groups. Group 1 (control) received drinking water only. The rats in Group 2 received in drinking water, 0.6 mL/kg/day benzene at the dose that was equal to one MPC dose (Hygiene standard 2.1.5.2280-07.2008. “Approximately permissible concentrations (APC) of chemicals in water, bodies of drinking, household, cultural-community water use,” 2008Hygiene standard, 2008Hygiene standard 2.1.5.2280-07.2008. “Approximately permissible concentrations (APC) of chemicals in water, bodies of drinking, household, cultural-community water use,” 2008). At days 45, 90 and 135 of treatments (OECD, 2009, 1998), subsets of rats were euthanized with ether anesthesia. The results of each set of experiments of Group 1 had no difference, thus all the animals at any duration, together constituted the control group. The experiments were conducted in accordance with the ethical standards and recommendations for humanization of work with laboratory animals, covered by the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe, 1985).

2.2. Immunological methods

The number of the cells, thymic index (thymus mass, mg/bdy mass, g) and splenic index (spleen mass, mg/bdy mass, g) were defined in blood, thymus and spleen of rats in accordance with the laboratory methods of experimental animal studies (Volchegorskiy et al., 2000).

For the immunophenotyping analysis, rats from each group were randomly selected. Immunophenotyping of splenocytes was conducted using monoclonal antibodies (‘ebioscience’, USA) against CD3, CD4, CD8 receptors. Percentage of CD3+, CD4+, CD8+ splenocytes was defined using the flow cytometer “FACS Canto II” with two lasers (“Becton Dickinson, USA”).

The splenocytes were cultured at 37 °C, 5% CO2 in RPMI-1640 medium, supplemented with 10% inactivated fetal calf serum, 2 mM glutamine and 80 μg/mL gentamycine. The cells were further stimulated with 5 μg/mL Con A and at 24 h, interferon-gamma (IFN-γ), IL-4, IL-6, IL-10 were assessed by enzyme linked immunosorbent assay (ELISA, “Bender MedSystems”, Austria). The absorbances were measured by using the photometer Multiskan (Labsystems, Finland).

The formation of cellular immunity was studied using the model of local delayed-type reaction. Thymus-dependent antigen response (ram’s erythrocytes) was studied by defining antibody producing cells in spleen by Jerne’s method and hemagglutinin content in hemagglutina-

tion reaction in blood serum (Volchegorskiy et al., 2000). Cell cycle and apoptosis of splenocytes were assessed using DNA fluorochrome staining method, followed by the cytofluorometry using the flow cytometer FACS Calibur (Sibirijak et al., 2008).

2.3. Statistical analysis

The results of the studies were processed using variation statistics methods with the help of software package “Microsoft Excel 7.0”, “STATISTICA 10.0”, including parametric (the Student’s test) and nonparametric analysis methods (the Mann-Whitney U test). The results are presented in the form of arithmetic mean value (Mean ± standard error of mean).

3. Results

3.1. Amount of nuclear cells in blood and lymphoid organs

The effect of benzene exposure on leukocytes and total number of lymphocytes were most pronounced at day 90. The number of leukocytes of the Wistar rats were decreased compared to the control group (6.3 ± 0.48 × 109 cell/mL and 10.10 ± 0.30 × 109 cell/mL, respectively). Similarly, the absolute number of lymphocytes of the animals were declined in comparison to the untreated group (5.08 ± 0.37 × 109 cell/mL and 8.87 ± 0.03 × 109 cell/mL, respectively).

Regarding lymphoid organs, the effect of benzene was expressed by the decrease in thymus mass, spleen mass and number of nuclear cells. The maximum decrease in thymus mass and the nuclear cells number in thymus was observed at Day 135, while the decline was more enhanced in spleen mass at Day 45, and in number of karyocytes in spleen at Day 90 (Table 1). Furthermore, thymic index, number of thymus nuclear cells corresponding to body mass (minimum at Day 135) and corresponding to the organ mass (minimum at Day 45), splenic index, and number of spleen nuclear cells corresponding to the body mass and corresponding to the spleen mass (minimum at Day 90) were decreased.

Thus, the dynamics of changes of nuclear cells number in blood and lymphoid organs of Wistar rats give evidence of the deleterious effects of benzene, which is expressed by leukopenia, decrease in lymphoid organs (thymus and spleen) mass and in the number of lymphoid organs cells. Additionally, thymic and splenic indexes and number of karyocytes in these organs, standardized according to body and organ mass were diminished. These alterations were more pronounced at Days 90 and 135.

3.2. Immunological parameters

Exposure to benzene led to a change in the pattern of T-lymphocyte sub-populations in the spleen of the rats (Table 2). The absolute number of CD3+ cells in the spleen decreased at Days 45, 90, and 135 (306 ± 19.16 × 106 cells, 255 ± 17.89 × 106 cells and 362 ± 24.04 × 106 cells, respectively) compared to the controls (452 ± 36.53 × 106 cells). Also, the absolute number of CD4+ cells was also decreased to 212.80 ± 20.02 × 106 cells, 185.23 ± 8.29 × 106 cells and 223.00 ± 22.92 × 106 cells at these timepoints, respectively, compared to the controls 339.42 ± 28.01 × 106 cells. The lowest absolute number of CD8+ cells was noted at Day 90.

The cytokine concentrations after Con A stimulation of the benzene exposed splenocytes are given in Table 3. Compared to the controls, while IL-4 production was significantly increased at all treatment groups, IL-6 levels were decreased and reached to a statistical significance at the Days 45 and 135.

Benzene was found to suppress the cell-mediated and humoral immune responses. It was expressed by the weakening of the delayed-type reaction intensity in the benzene treated rats, 65.71 ± 10.14 mg.
A.V. Karaulov et al.
Toxicology Letters 275 (2017) 1–5

The effect of benzene on the parameters of thymus mass, spleen mass, number of karyocytes, thymic index and splenic index of Wistar rats.

| Control group (n = 47) | Benzene exposed rats |
|-----------------------|----------------------|
|                       | 45 days (n = 26)     | 90 days (n = 21) | 135 days (n = 12) |
| Body mass (g)         | 316.00 ± 8.66        | 269.79 ± 10.59  | 351.10 ± 8.30**  | 323.33 ± 6.38*** |
| Thymus mass (mg)      | 246.74 ± 9.30        | 238.10 ± 8.77   | 215.04 ± 11.15   | 169.75 ± 11.03*** |
| Thymus karyocytes (x10^6) | -434.23 ± 22.91 | 314.00 ± 20.58  | 348.17 ± 14.62   | 267.58 ± 22.15*** |
| Thymic index (mg/g)   | 0.82 ± 0.05          | 0.86 ± 0.04     | 0.62 ± 0.03      | 0.54 ± 0.04      |
| Thymus nuclear cells/body mass (x10^6/g) | 1.45 ± 0.10          | 1.18 ± 0.09     | 1.00 ± 0.04      | 0.83 ± 0.07      |
| Spleen mass (mg)      | 1041.62 ± 22.54      | 950.50 ± 33.68  | 977.23 ± 30.97   | 963.83 ± 45.60   |
| Spleen karyocytes (x10^6) | -1030.85 ± 29.61 | 851.97 ± 36.58  | 623.13 ± 46.44*** | -643.08 ± 47.88*** |
| Spleenic index (mg/g) | 3.40 ± 0.15          | 3.52 ± 0.17     | 2.81 ± 0.11      | 3.01 ± 0.18      |
| Spleen nuclear cells/body mass (x10^6/g) | 3.40 ± 0.18          | 3.12 ± 0.17     | 1.77 ± 0.14      | 2.00 ± 0.15      |
| Spleen lymphocytes/spleen mass (x10^6/mg) | 1.01 ± 0.03          | 0.90 ± 0.04     | 0.63 ± 0.04      | 0.69 ± 0.07      |

The amount of cells were measured in the Boiden camera with following the recalculation per whole organ.

* * * p < 0.05, statistically significant differences between benzene exposed vs control groups.
** ** ** p < 0.05, statistically significant differences between 45 and 90 days or 45 and 135 days.
*** *** *** p < 0.05, statistically significant differences between 90 and 135 days.

Exposure to benzene, which is classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen, has been concluded to cause hematologic malignancies, based on results from several case-control and occupational cohort studies in human populations ("mono100F-24.pdf," n.d.). In addition, a variety of other adverse health effects associated with benzene have been observed in animal and human studies (Wilbur et al., 2008). Despite the meta-analysis revealed that benzene exposure increased the risk of hematologic disorders, the lack of consistent exposure categories with the comparable criteria precludes the risk assessment by exposure time and exposure level ("mono100F-24.pdf," n.d.). Recent comprehensive evaluation by IARC concluded that there is evidence in humans for a positive association between benzene and non-Hodgkin lymphoma (NHL) overall, as well as for acute lymphocytic leukemia and multiple myeloma ("mono100F-24.pdf," n.d.). Indeed, the effects of benzene metabolites on the functions of the immune cells can vary depending on the cell type and the stimulus used to induce the immune response (Geiselhart et al., 1997). Higher levels of passive benzene exposure are associated with increased NHL risk across various lag periods (Switchenho et al., 2016). Although benzene can affect all the major peripheral blood elements, the absolute lymphocyte count is the most sensitive indicator of benzene-induced hematotoxicity (McMurry et al., 1994; Robinson et al., 1997; Rothman et al., 1996). In our study the most pronounced decrease in lymphocytes was observed at 90ths days. In fact, lymphocyte proliferation is important for prolonged immune responses. Therefore, we determined whether benzene could modulate lymphocyte proliferation induced by the T cell mitogen Con A. Lymphocyte proliferation induced by the T cell mitogen Con A shows that benzene, concentration-dependently suppressed both viability and T-lymphocyte sub-populations in the spleen. Eventually, Con A-induced T cell proliferation was greatly suppressed. Furthermore, the absolute number of CD4+ T cells and CD3+ cells in the spleen is decreased at all time-points of this study.

In this respect, the inverse association of benzene exposure with levels of CD4+ T cells is particularly notable given the established relationship between immune deficiency and various immune disorders, as well as NHL risk (Grulich et al., 2007). A cross-sectional study of occupationally benzene-exposed workers demonstrated significant decreases in lymphocyte cell counts, CD4+ T cells and CD4+/CD8+ ratio. These changes have been associated with an increased risk of NHL (Lan et al., 2004). In a meta-analysis, Smith et al. concluded that 40 of 43 studies show some evidences related to increase in risk of NHL. Furthermore, the results of 23 studies shown statistically significant associations between NHL risk and benzene exposure (Smith et al., 2007). Eventually, compared with unexposed individuals, exposed subjects have significant risk of NHL which is depend on increasing

Table 2
The effect of benzene on the level of T-lymphocyte subpopulation in spleen.

| Control (n = 24) | Benzene exposed rats |
|------------------|----------------------|
|                  | 45 days (n = 10)     | 90 days (n = 13) | 135 days (n = 6) |
| Splenocyte Number | 900.13 ± 68.27       | 731.40 ± 53.38  | 525.23 ± 15.30*** | 732.83 ± 51.02*** |
| CD4+ cells (%)    | 37.50 ± 0.87         | 29.05 ± 1.45    | 35.20 ± 0.99    | 30.28 ± 1.86*** |
| CD4+ cells (number of cells) | 339.42 ± 28.01 | 212.80 ± 20.02  | 185.23 ± 8.29*  | 223.00 ± 22.92*** |
| CD8+ cells (%)    | 14.6 ± 1.08          | 18.74 ± 0.82    | 16.08 ± 2.00    | 17.03 ± 1.68*** |
| CD8+ cells (number of cells) | 137.13 ± 18.59 | 136.68 ± 11.75  | 84.46 ± 11.01*** | 126.50 ± 18.24*** |
| CD4+ and CD8+ cells | 2.90 ± 0.21         | 1.57 ± 0.10     | 2.64 ± 0.32     | 1.87 ± 0.21*** |

* * * p < 0.05, statistically significant differences between benzene exposed vs control groups.
** ** ** p < 0.05, statistically significant differences between 45 and 90 days or 45 and 135 days.
*** *** *** p < 0.05, statistically significant differences between 90 and 135 days.
duration of exposure and increasing cumulative exposure levels of benzene. Thereby, the highest duration and cumulative exposure of benzene shows a significantly higher association with NHL (Bassig et al., 2015). In our study, both absolute number of CD4 + T cell counts and percentages of CD4 + T cell significantly decreased in comparison to controls at 45, 90 and 135-day time-points, but only at 90-day exposure period decreasing in CD4 + T cell percentage did not reach to statistical significance. Virtually, primed human peripheral blood mononuclear cells (PBMC) and purified CD4 + or CD8 + T cells were used in a cell-mediated cytotoxicity assays (Yi et al., 1999). The aims of CD8- or CD8-mediated lysis are also different. CD8 + CTL recognizes and kills their targets, whereas CD4 + CTL plays an immunomodulatory role through the elimination of activated myeloid and lymphoid cells during and upon completion of immune response (Hahn et al., 1995). CD4 + T cells can express cytotoxic activity. The ability of CD4 T cells to acquire cytotoxic functions is associated with the possibility turning into T helper type 1 (Th1) cells, but not with the Th2-phenotype (Tian et al., 2016). In our study, Th2-type cytokine, IL-4 significantly increased, whereas IL-6 decreased (Table 3). In this context, CD4 + T cells could not exert cytotoxicity in benzene exposed subjects. As in previous studies, we did not observe a consistent variation in percentages and absolute number of CD8 + T cells of benzene-exposed animals. Nevertheless, percentages of CD8 + T cells tended to increase. However, significant decreases were found in CD4/CD8 ratios at 45 and 135-day benzene exposure. Although, multivariate analysis revealed that only low absolute CD4 + T cell counts proved to be a significant predictor of shorter overall survival in patients with lymphoma, also low CD4/CD8 ratio are associated with unfavorable overall survival. (He et al., 2016; Zhang et al., 2016). In our study, IFN-γ production of Con A-induced splenocytes tended to increase, however, average concentrations of IFN-γ did not reach to statistically significant levels in benzene exposed animals. Decreasing percentages and absolute number of CD4 + T cell and reduced CD4/CD8 ratio supported these findings. In various studies, despite benzene toxicity, no significant difference could be observed in CD8 + T cell counts when compared with controls (Shen et al., 2006; Uzma et al., 2010). Repeated administration of benzene produces leukopenia, lymphocytopenia and an increased number of nucleated cells in the bone marrow and significantly decreased organ weights of thymus and spleen in rats (Pandya et al., 1986). According to findings of Rozen et al., no corresponding increase in splenic cells is observed in benzene-exposed mice (Rozen and Snyder, 1985). In accordance with these findings, the dynamics of changes in nuclear cells number in blood and lymphoid tissues of Wistar rats demonstrated the evidence of benzene effects such as decreased thymic and splenic indexes and reduced number of karyocytes.

In our study, Con A induced production of the cytokines by splenocytes was characterized by the increased level of IL-4 and decreased IL-6 levels. Actually, hydroquinone (HQ) and benzoquinone, two important chemicals implicated in benzene toxicity, on the release of IL-4 from human basophils. These metabolites inhibit the release of proinflammatory mediators and Th2-promoting cytokines from basophils (Borreli et al., 2010). HQs enhance Th2 response-mediated allergic diseases via blockade of IFN-γ production in Th1 cells, with simultaneous increase in IL-4 production of CD4 + T cells (Kim et al., 2005; Lee et al., 2002). On the other hand, HQ inhibits IFN-γ secretion by decreasing the mRNA expression of IFN-γ in effector CD4 + T cells and Th1-differentiated CD4 + T cells at the transcriptional level (Choi et al., 2008).

Accumulation of benzene metabolites in bone marrow and lymphoid organs lead to hypoplasia of central and peripheral immune organs. This phenomenon causes hypocellularity in spleen and thymus, as in our study. It may be caused by the decreased number of pluripotent stem cells or clear cells, due to the direct cytotoxic activity of benzene (or its metabolites, especially 1,4-benzoquinone) (Snyder, 2002). The high lymphoid tissue sensitivity to benzene is caused by a prolonged cell cycle duration, aerobic metabolism, and a high rate of oxidative phosphorylation of lymphoid tissues. Besides, it was stated that benzene has an ability to block cell division at the G2/M phase during DNA replication and mitotic cycle (Schoenfeld and Witz, 1999). DNA damage in the blood cells and organs is also found to vary directly with benzene exposure, in both a dose-dependent and time-dependent manner. Benzene exposure results in significant DNA damage in the T and B lymphocytes, bone marrow, spleens, and livers of rats (Lee et al., 2005).

5. Conclusion
The present study showed that total WBCs, lymphocytes and T cells significantly decreased with increasing benzene exposure. In addition to the alteration of cytokine production by stimulated splenocytes, lymphocyte subset analysis revealed significantly decreased CD4 + T cells, CD4 + /CD8 + ratio and CD3 + T cells. Forty-five-day of exposure to benzene appeared to be the most sensitive time point for evaluating benzene cytotoxicity. These changes were similar to hematological changes seen at high levels of long-term benzene exposure. The identified biomarkers were positively correlated to benzene toxicity and indicated increased immune responses, which are seen in NHL with respect to 45-day benzene exposure despite the absence of lymphoma.

Author disclosure statement
All authors disclose that they have any financial or personal relationships with other people or organizations. There is no direct financial interest in the subject matter or materials discussed in the manuscript that could inappropriately influence the work submitted. No competing financial interests exist.

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References

Agency for Toxic Substances and Disease Registry, 2007. Toxicological Profile for Benzene. Atlanta, Georgia.

Bassig, B.A., Friesen, M.C., Vermeulen, R., Shu, X.-O., Purdie, M.P., Stewart, P.A., Xiang, Y.-B., Chow, W.-H., Zheng, T., Ji, B.-T., Yang, G., Linet, M.S., Hu, W., Zhang, H., Zheng, W., Guo, Y.-T., Rothman, N., Lan, Q., 2015. Occupational exposure to benzene and non-Hodgkin lymphoma in a population-based cohort: the Shanghai women's health study. Environ. Health Perpect. 123, 971–977. http://dx.doi.org/10.1289/ehp.1408207.

Benzene, 1982. (IARC Summary & Evaluation, Volume 29) [WWW Document], n.d. URL http://www.inchem.org/documents/iarc/vol29/benzene.html (Accessed 2.17.17).

Borrelli, I., Loffreda, S., Staziuna, I.I., Frattini, A., Bergamaschi, A., Marone, G., Triggiani, M., 2010. Benzene metabolites inhibit the release of proinflammatory mediator and cytokines from basophilic. Int. J. Immunopathol. Pharmacol. 23, 737–744.

Choi, J.M., Cho, Y.-C., Cho, W.J., Kim, T.S., Kang, B.Y., 2008. Hydroquinone, a major component in cigarette smoke, reduces IFN-gamma production in antigen-primed lymphocytes. Arch. Pharm. Res. 31, 337–341. http://dx.doi.org/10.1007/s12272-007-0116-1.

Costa, C., Onzagli, E., Gangemi, S., Schembri, F., Giambò, F., Androustopoulos, V., Tsatsakis, A., Fenga, C., 2016. Molecular biomarkers of oxidative stress and role of dietary factors in gasoline station attendants. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 90, 30–35. http://dx.doi.org/10.1016/j.fct.2016.01.017.

Council of Europe, 1985. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Strasbourg.

Fenga, C., Gangemi, S., Giambò, F., Taisimpikou, C., Golokhvast, K., Tsatsakis, A., Costa, C., 2016. Low-dose occupational exposure to benzene and signal transduction pathways involved in the regulation of cellular oxidative stress. Life Sci. 147, 67–70. http://dx.doi.org/10.1016/j.lfs.2015.12.025.

Geiselhart, L.A., Christian, T., Minnear, F., Freed, B.M., 1997. The cigarette tar component β-benzoquinone blocks T-lymphocyte activation by inhibiting interleukin-2 production, but not CD25, ICAM-1, or LFA-1 expression. Toxicol. Appl. Pharmacol. 143, 30–40. http://dx.doi.org/10.1016/S0041-008X(96)00001-6.

Grujic, A.E., Vajdic, C.M., Cozen, W., 2007. Altered immunity as a risk factor for non-Hodgkin lymphoma. Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 16, 405–408. http://dx.doi.org/10.1158/1055-9965.EPI-06-1070.

Hahn, S., Kirkeleit, J., Ulvestad, E., Riise, T., Bråtveit, M., Moen, B.E., 2006. Acute suppression of hematotoxicity by an interferon inducer (6MFA). Toxicology 219, 295–302. http://dx.doi.org/10.1016/j.tox.2005.06.009.

Hygiene standard 2.1.5, 2008. 2008. Approximately Permissible Concentrations (APC) of Chemicals in Water Bodies of Drinking, Household, Cultural-Community Water Use. Rospotrebnadzor Publications, Moscow.

Kim, E., Kang, B.Y., Kim, T.S., 2005. Inhibition of interleukin-12 production in mouse B lymphocytes, bone marrow, spleens, and livers of rats exposed to benzene. Inhal. Toxicol. 17, 401–408. http://dx.doi.org/10.1080/0895705090295259.

Lindstrom, A.B., YeoWell-O'Connell, K., Waidyanatha, S., Golding, B.T., Tomoro-Velez, R., Rappaport, S.M., 1997. Measurement of benzene oxide in the blood of rats following administration of benzene. Carcinogenesis 18, 1637–1641.