ISSN : 2321-9602

Indo-American Journal of Agricultural and Veterinary Sciences




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Indo-Am. J. Agric. & Vet. Sci., 2013

ISSN 2321–9602 www.iajavs.com
Vol. 1, Issuse 2 June 2013

Indo-American Journal of Agricultural and Veterinary Sciences

Toxicity of benzene: a synopsis of research in humans and animals

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ABSTRACT:

A large population of humans is exposed to benzene from various occupational and environmental sources. Benzene is an established human and animal carcinogen. Exposure to benzene has been associated with leukaemia in humans and several types of malignancies in animals. The exact mechanism of benzene-induced toxicity is poorly understood. It is believed that benzene exerts its adverse effects by metabolic activation to toxic metabo- lites. Certain benzene metabolites are genotoxic andmutagenic. This consolidated short-review is composed of human and animal studies to summarize the adverse effects of benzene with special reference to molecular mechanisms involved in benzene-induced toxicity. *Human &*

Key words: benzene; chromosomal aberrations; cytotoxicity; geno-toxicity; health hazards; mutations; sister chromatid

Introduction

Benzene is a key intermediate in the production of several other compounds with important industrial and medical applications. It is a top-notch solvent, making it useful in several manufacturing processes. Cigarette smoke and gasoline both contain benzene. Numerous people all around the world are subjected to benzene pollution either in their workplaces or their homes. The carcinogenic effects of benzene on both humans and animals have been established. Low-dose, chronic benzene exposure has been linked to aplastic anemia and leukemia. Mucous membrane irritation, agitation, convulsions, excitation, depression, and death from respiratory failure are all acute consequences of benzene exposure. Benzene is very harmful to the haematopoietic system. This short summary is aimed to compile various aspects of benzene toxicity in humans and experimental animals by incorporating the relevant studies beginning from the decades old historical contributions to current insights on benzene toxicity.Human toxicological studies *Occupational and environmental exposure*

A large number of industrial workers from petrole- um, rubber, paint, shoe making, printing, solvent and other chemical industries are occupationally exposed to benzene[1–4] Moreover, the individuals working at the petrol filling stations, tanker crew,motor mechanic and traffic policemen are also at apotential risk to benzene exposure occupationally.[5–9] The major sources of environmental exposure to benzene include active and passive smoking, auto exhaust and driving or riding in automobiles.[10–12] Jermann *et al.*[13] found high levels of benzene in theblood of children from the urban areas with high traffic density as compared with the children from rural areas. Although inhalation is the main route

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of benzene exposure because of its appreciable vapour pressure at ambient temperature it may also get a route through contaminated drinking water[14,15] and dermal absorption.[16,17] Nakai *et al.*[18] have suggested that use of sunscreens may signifi- cantly enhance the absorption of benzene into the skin. Accidents like oil fires may also significantly enhance the environmental exposure of benzene.[19] Benzene contamination of soil may also occur due to oil production facilities and coastal refineries.[20,21]

Human health hazards

Chronic exposure of humans to benzene is associat- ed with blood disorders including aplastic anaemia[22–24] and leukaemia.[25–28] Aplastic anaemia results from bone marrow toxicity of benzene caus- ing progressive decrease in erythrocytes, thrombo- cytes and each of the various types of leukocytes. The incidence of aplastic anaemia at high level of benzene exposure (>100 ppm) has been reported to be 1/100, which drops to around 1/10 000 at an exposure level of 10–20 ppm.[22] Working atmospher- ic concentrations of more than 5 ppm of benzene significantly reduced the haemoglobin level and mean corpuscular haemoglobin concentration in occupationally exposed workers.[29] However, even low levels of benzene exposure (<5 ppm) may also result in haematological suppression.[4] Molinini *et al.*[3] have suggested that synergistic effects of other environmental contaminants may promote myelo- toxic effect of low level of benzene exposure. Baak *et al.*[24] also reported a case of aplastic anaemia induced by low-level benzene exposure. On the other hand, Collins *et al.*[30] found the benzene expo- sure of 0.55 ppm to be safe without any indication of haematotoxicity.

Leukaemia is a neoplastic type of blood dyscrasia associated with benzene exposure.[31,32] The benzene- induced leukaemia is acute myeloid leukaemia that is characterized by increased number of cells mor- phologically similar with myeloblasts. Aksoy *et al.*[28] reported high incidence of leukaemia in shoe work- ers occupationally exposed to benzene, whereas Hunting *et al.*[33] observed high haematopoietic can- cer mortality in motor vehicle mechanics. Nordlinder and Jarvholm[34] found a close association between leukaemia and the density of motor vehicles (>20 cars/km^2). The duration of exposure to benzene was found to be closely related with the risk of leukaemia in petroleum distribution workers.[35] A minimum critical concentration of 50–60 ppm of benzene may be required for the risk of leukaemia in pliofilm workers.[36] Xu *et al.*[37] reported an increased risk of spontaneous abortion for the woman

frequently exposed to petrochemicals including ben- zene. As an acute poison, benzene produces narcot- ic effects comparable to those of toluene. The human oral lethal dose would probably be between 50 to 500 mg/kg.[38] Human inhalation of about 20 000 ppm (2% in air) was fatal in 5–10 min.[39] The acute symp- toms of benzene exposure may include headache, nausea, vertigo, fatigue and dizziness.[6]

Cytotoxicity

Snyder *et al.*[40] pointed out that benzene and its metabolites do not function well as mutagens, but are highly clastogenic, producing chromosomal aberrations, sister chromatid exchange (SCE) and micronuclei. An increased incidence of chromoso- mal aberrations has been reported in workers exposed to 25–150 ppm benzene for 1–25 years as compared with general population.[41] Killian and Daniel[42] observed that long-term exposure to even below 10 ppm levels of benzene caused about two-fold increase in chromosomal breaks and a three-fold increase in rings and dicentric chromo- somes as compared with controls. Sarto *et al.*[43] found an increase in chromosome aberrations, but no increase in SCE among 22 workers exposed to 0.2
–12.4 ppm benzene for about 11 years, as compared with controls matched for sex, age, smoking habits and site of residence. Yardley-Jones *et al.*[44] observed a significant increase in chromosomal aberrations, whereas no significant change in SCE frequencies was noticed in 60 workers exposed to 100 ppm benzene. Erexson *et al.*[45] studied the effect of benzene and its metabolites on SCE induction in human lymphocytes. They observed that catechol was highly potent for inducing SCE, follow by 1,4-benzoquinone > hydroquinone > 1,2,4-benzen-etriol > phenol > benzene. On the other hand, ben- zene exposure at 1–9 ppm for 1–20 years or 3–50 ppm for 2–12 years failed to produce any significant effect on chromosomal aberrations and SCE in female workers.[46] The occupational exposure of benzene failed to increase SCE frequency in 36 workers employed in shoe industry.[47] Bukvic *et al.*[48] concluded that the occurrence of SCE was signifi- cantly associated with age and smoking habits and not with the length of employment at gasoline station. There was no significant difference in the frequency of SCE between traffic wardens and office workers; whereas the same study revealed highly

ISSN 2321–9602 www.iajavs.com
Vol. 1, Issuse 2 June 2013

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significant increase in SCE among smokers.[49] On the contrary Celi and Akbas[50] observed significant difference in SCE in gasoline station workers as compared with control individuals; there was no interaction between cigarette smoking and benzene exposure for SCE values.

Ding *et al.*[51] reported a significant increase in hypodiploid and hyperdiploid cells in benzene-exposed patients of either sex. The chromosome deletions in the hypodiploid cells involved groups C, E and G chromosomes whereas chromosome gains in the hyperdiploid cells involved groups C and E. Although high concentrations of benzene are associated with increased levels of cytogenic indices of genotoxicity in exposed workers, the concentrations below TLV do not produce any measur- able cytogenic damage in humans. The incidence of dicentric chromosomes in the shoe industry work- ers has been found to be significantly higher than control group.[47] Benzene decreases peripheral white blood cell and platelet counts and specifically low- ers T-cells, B-cells, NK-cells and granulocytes; how- ever, benzene's lymphotoxicity is not mediated by diminished thymus function.[52] On the other hand, human bone marrow CD34(+) haematopoietic pro- genitor cells are sensitive targets for benzoquinone (a metabolite of benzene) toxicity that utilizes the p53 pathway for protection against benzoquinone-induced DNA damage.[53] The inhibition of topoisomerase II (a key enzyme of DNA replication) has also been linked to benzene-induced cytotoxicity and leukemogenesis.[54] Chung and Kim[55] have reported that treatment with benzene metabolites resulted in the induction of monosomy in human lymphocytes in a concentration-dependent manner. Rothman *et al.*[56] used glycophorin A (GPA) gene loss mutation assay to evaluate the nature of DNA damage produced by benzene exposed workers. A significant increase in GPA variant cell frequency was observed in benzene exposed workers as com- pared with control subjects, suggesting the role of chromosomal damage and mitotic recombination in benzene-induced leukaemia. Polymorphisms in the genes involved in benzene metabolism influence the susceptibility of individuals to chromosomal aberrations in relation to benzene exposure.[57] NADPH:quinone oxidoreductase 1 (NQO1) performs multiple functions including cellular redox balance, scavenging of ODFR, stabilization of p53 and stabilization of microtubules; the NQO1*2 polymorphism predisposes to benzene toxicity and to various forms of leukaemia.[58]

Animal toxicological studies

Subchronic toxicity

Data from animal models are frequently used in risk assessment of benzene. Animal studies have shown that mice are more sensitive to benzene toxicity than rats[59,60] hence, they are preferred fortoxicological studies. Faiola *et al.*[61] observed that male mice were more susceptible to benzene toxicity following the exposure of up to 100 ppm benzenefor 6 h/day, five days/week for two weeks. Haematotoxity was evident in all male mice but not seen in female mice.[61] It is plausible that the enzymes that activate and detoxify benzene are like- ly the genetic determinants of benzene's toxicity. Bauer *et al.*[62] found that the male mice deficient in microsomal epoxide hydrolase (mEH −/−) did not show any significant haematotoxicity or myelotoxi- city at the highest benzene exposure (100 ppm). On the other hand, deficiency of NQO1 (−/− genotype) predisposes both male and female mice to benzene- induced toxicity.[63] Although cytochrome P450 2E1 (CYP2E1) dependent metabolic activation has been implicated in benzene's toxicity[64] this pathway failed to define the strain-specific differences in tumour response of the haematopoietic system of mice.[65] Ward *et al.*[66] reported that exposure of 300 ppm benzene, 6 h/day, five days/week for 13 weeks produced significant haematological changes including decreased haematocrit, total haemoglobin, erythrocyte/leukocyte count, platelet count and myeloid:erythrocyte ratio in CD-1 mice. However, the rats exposed to the similar doses of benzene showed only a decrease in lymphocyte count and increase in neutrophil count. Exposure of mice to 300 ppm benzene (6 h/day for 10 days) produced long-lasting haematotoxic effects.[67] Farris *et al.*[68] observed that mice exposed to 100–200 ppm ben- zene for eight weeks showed persistent reductions in femoral B-, splenic T- and B- and Thymic T- lymphocytes. The target cells for benzene haematopoietic toxicity include pluripotent stem cells, the colony-forming cell units in the spleen and the progenitor cells for granulocytes and macrophages. Rozen and Snyder[69] reported a sig- nificant decrease in B-lymphocytes in bone marrow and spleen and T-lymphocytes in thymus and spleen of C57BL mice exposed to 300 ppm benzene, 6 h/day, five day/week for 115 exposures. The expo- sure of 50–200 ppm benzene, 6 h/day for 7 to 14 days depressed the ratios and absolute numbers of T- and B-lymphocytes in blood and spleen of BALB/C mice.[70] A significant depression in colony-forming units in B-lymphocytes was observed in C57 BL mice after inhalation of 10 ppm of benzene 6 h/day for six days, whereas the dose of 31 ppm also resulted in depressed

ISSN 2321–9602 www.iajavs.com
Vol. 1, Issuse 2 June 2013

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blastogenesis of T-lymphocytes.[71] Cronkite *et al.*[72] reported sig- nificant reduction in bone marrow cellularity in CBA/CA mice following benzene exposure at
100 ppm or greater, 6 h/day, five day/week for

Carcinogenicity
Maltoni and Scarnato[73] clearly demonstrated the carcinogenicity of benzene in experimental animals. Benzene is a potent carcinogen in rodents when administered by inhalation or ingestion, producing carcinomas of the Zymbal gland, oral cavity, nasal cavities, skin, stomach, mammary glands, lungs and liver. Thus, benzene is considered as a multipoten- tial carcinogen that produces cancers in several organs of various species of animals by different routes of administration.[73–76] Various concentra- tions of benzene, route and duration of exposure have been used in long-term studies on benzene-induced carcinogenesis in mice.[77–79] Snyder *et al.*[80] reported significantly higher incidence of tumours in C57BL mice inhaled 300 ppm benzene for 6 h/day, five days/week for two years. Oral adminis- tration of benzene in the doses of 50 and 250 mg/kg,
4 to 5 days/week for 52 weeks caused a dose-dependent increase in total cancers in rats. Cronkite and coworkers[77,81] reported comparatively high incidence of benzene-induced leukemia in male C57BL/6 and CBA/CA mice than females following the exposure of 100–300 ppm benzene, 6 h/day, five days/week for 16 weeks.
Developmental toxicity
Kuna and Kapp[82] observed significant reduction in fetal body weight and crown-to-sump distance as well as developmental delay following the exposure of benzene (10, 50 and 500 ppm 7 h/day) to pregnant rats during 6–15 days of gestation. They suggested benzene-induced fetotoxicity in rats at 50 and
500 ppm, and teratogenecity at 500 ppm. Coate *et al.*[83] also reported reduction in fetal body weight due to maternal exposure of benzene (100 ppm, 6 h/day) on days 6–15 of gestation, however, no terato- genecity was observed. Twenty-four hours exposure to 308 ppm benzene during 6–15 days of gestation has been shown to retard skeletal development in mouse fetus and spontaneous abortions in rabbits.[84] Whereas, administration of benzene to pregnant experimental animals at concentrations, which did not cause maternal toxicity also failed to produce any remarkable developmental effects in the fetus.[85]

Genotoxicity
Mutations Several investigators have observed negative findings for benzene-induced mutagenesis using *Salmonella typhimurium* gene mutation assay.[86,87] However, McCarroll *et al.*[88] reported pos- itive result using a microsuspension assay with hepatic microsomal activation resulting in an increase in the number of revertants in *S. typhimurium* strain TA100. The growth of DNA repair deficient

Escherichia coli strain WP100 was inhibited by ben- zene exposure, but no such effect was observed in repair proficient strains.[89] Rossman *et al.*[90] exam- ined the mutagenic potential of several metabolites of benzene using *E. coli* WP2s (lambda) as a target and concluded that only t,t-muconic acid, which is metabolized to a diepoxide may be the ultimate mutagen and possibly the ultimate carcinogen. Benzene was reported to be negative in *Saccharomyces cerevisiae* gene conversion and mitotic crossing-over assays,[91] however, it was considered mutagenic for *S. cerevisiae* strains D61- M and D6.[92] Recently, p-benzoquinone-induced deletion recombination in *S. cerevisiae* at a dose of 300-fold lower than any of the other metabolites of benzene suggested that it might be responsible for much of benzene's genotoxicity.[93] The metabolite of benzene, p-benzoquinone readily forms adducts with the nucleotides leading to deletions in the tran-scription products at the site of damage.[94] Whereas DNA adducts subject to excision repair were not formed by benzene or its metabolites, as demon- strated using excision repair deficient strain of
S. cerevisiae.[93] Studies on Drosophila have shown no evidence for mutagenic response to benzene exposure.[95,96] Moreover, studies on cultured mam- malian cells have also been inconclusive regarding an association between point mutation and benzene exposure.[97] Ward *et al.*[98] noticed that subchronic exposure to benzene at levels below the occupation- al safety permitted exposure limit may induce gene mutations in lymphocytes of CD-1 mice. Significant increases in lacI mutations were observed in the spleen and bone marrow of benzene treated mice using the standardized lambda/lacI transgenic mouse mutation assay.[99]

Chromosomal aberrations Benzene exposure has been shown to induce chromosomal structural changes and aneuploidy in cultured mammalian cells. Howard *et al.*[100] reported chromosomal aber- rations in cultured human lymphocytes after 3 h incubation with 9–88 μg benzene/mL, with or with- out S9 activation. Benzene concentrations of 100 and 1100 μg/mL, with S9 activation produced aber- rations in Chinese

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hamster ovary cells and lung fibroblasts, respectively.[101] Eastmond[102] suggested that the chromosomal aberrations induced by the quinone metabolites of benzene may play a rolein the carcinogenic effects of the parent compound. The metabolic activation of benzene is necessary for the induction of sister-chromatid exchange (SCE) in cultured human lymphocytes, whereas its metabo- lites cathecol and hydroquinone are potent SCE inducers.[103] Glutathione has been suggested to play

an important role in protecting against benzene-induced SCE formation by conjugating with toxic metabolites.[104] Tice *et al.*[105] reported a dose-dependent increase in SCE following benzene exposure where the mice with male sex and younger age were more sensitive to SCE formation.

DNA damage Although benzene failed to exhibit genotoxicity in tests for unscheduled DNA synthesis (UDS) in cultured primary rat hepatocytes, an asso- ciation between benzene exposure and increased UDS has been reported by Glauert *et al.*[106] Cell transforming and genotoxic effects of benzene and its metabolites using cultured mammalian cells have been reported.[107] Pellack-Walker and Blumer[108] observed that exposure to 1.0 mmol of benzene, phenol or catechol or 0.1 mmol of hydro- quinone failed to show any single-strand breaks in the DNA of mouse lymphoma cells, however, 70% DNA breakage was observed with p-benzoquinone or 1,2,4-benzenetriol at a concentration of 6 μmol exposed for 3 and 60 min, respectively. Benzene metabolites, hydroquinone and benzoquinone are highly potent to induce DNA strand breakage in chi- nese hamster ovary cells.[109] The enhanced produc- tion of oxygen-derived free radicals (ODFR) by redox-cycling of phenoxy radicals has been impli- cated in the oxidative damage of DNA.[110] Rao[111] suggested glutationyl hydroquinone to be a possible toxic metabolite of benzene with pro-oxidant prop- erty for the degradation of DNA. The involvement of ODFR in benzoquinone-induced DNA damage has been observed using the comet assay of peripheral blood mononuclear cells.[112] Recent studies have shown that modulation of topoisomerase II enzyme by benzene's metabolites play a key role in benzene- induced DNA damage.[113,114]

Neoplasia Although benzene was not found to be mutagenic in cultured Balb/C 3T3 mouse fibroblastsand in simian adenovirus-transformed Syrian hamster embryo (SHE) cells, it was considered to be mutagenic in SHE cells using morphologically transformed colonies as a marker.[115] Alteration in gene expression in Swiss mouse spleen lympho- cytes exposed to benzene or its metabolites hydro- quinone and p-benzoquinone has been reported.[116] The concentration of 10–20 μmol of both hydro-quinone and p-benzoquinone caused 50% inhibi-tion in RNA synthesis. The inhibition of T-cell proliferation and reduced production of interleukin- 2 by p-benzoquinone was suggested to be a possible cause of benzene-induced aplastic anemia.[116] Bioactivation of benzene causes increased genera- tion of ODFR, which in turn activates the oncogene c-myb leading to the formation of leukemic cells.[117] Studies on C57BL/6 normal and p53 knockout mice have shown that benzene suppresses the cell cycle by p53 mediated overexpression of p21, a cyclin-dependent kinase inhibitor, resulting in dynamic change of haemopoiesis during and after benzene exposure.[118] Gene expression profiles in bone mar-row cells from C57BL/6 p53 +/+ and isogenic p53 +/– mice chronically exposed for 15 weeks to 100 ppm of inhaled benzene revealed that p53 homozygous mice expressed significantly higher levels of a majority of key genes involved in p53-regulated DNA damage response pathway compared with p53 heterozygous bone marrow cells.[119] The mice lacking expression of NQO1 and NQO2 protein showed myelogenous hyperplasia of the bone marrow and increased granulocytes in the peripher- al blood accompanied by decreased apoptosis.[120] Short-term exposure of NQO1–/– mice to benzene demonstrated substantially greater benzene-induced toxicity as compared with wild type mice.[120] Nitric oxide has also been suggested to be an importantmediator of benzene-induced bone marrow suppression and haematopoietic impairment in mice.[121]

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