

937 Agency for Toxic Substances and Disease Registry (ATSDR)
toxicological profile for benzene

<http://www.atsdr.cdc.gov/ToxProfiles/tp3.pdf>

In vitro toxicology – p. 12, 18, 20, 89, 132, 146-148, 152-154, 159, 161, 163, 168, 169, 171, 173, 225

Dermal/inhalation carcinogenicity – p. 15, 16, 97-104, 137, 140-141, 224, 311

Cardiovascular toxicity – p. 12, 73, 105

Inhalation studies – p. 16, 21-26, 30-104, 155-158, 161, 163-165, 174, 175-177, 188, 223, 227, 228, 311

Reprotoxicity & development toxicity – p. 6, 92-97, 135-136, 140, 225-227

Summary of Health Effects

The carcinogenicity of benzene is well documented in exposed workers. Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute myelogenous leukemia (AML). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power, but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor.

In vivo and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic. Chromosomal aberrations (hypo- and hyperdiploidy, deletions, breaks, and gaps) in peripheral lymphocytes and bone marrow cells are the predominant effects seen in humans.

Damage to both the humoral and cellular components of the immune system has been known to occur in humans following inhalation exposure. This is manifested by decreased levels of antibodies and decreased levels of leukocytes in workers. Animal data support these findings.

The most characteristic systemic effect resulting from intermediate and chronic benzene exposure is arrested development of blood cells. Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. A common clinical finding in benzene hematotoxicity is cytopenia, which is a decrease in various cellular elements of the circulating blood manifested as anemia, leukopenia, or thrombocytopenia in humans and in animals. Benzene-associated cytopenias vary and may involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood.

Benzene also causes a life-threatening disorder called aplastic anemia in humans and animals. This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow, leading to fibrosis, an irreversible replacement of bone marrow. Benzene has also been associated with acute non-lymphocytic leukemia in humans, and aplastic anemia may be an early indicator of developing acute non-lymphocytic leukemia in some cases.

Limited information is available on other systemic effects reported in humans and is associated with high-level benzene exposure. Respiratory effects have been noted after acute exposure of humans to benzene vapors. Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the cause of death in fatal exposures to benzene vapor. Gastrointestinal effects have been noted in humans after fatal inhalation exposure (congestive gastritis), and ingestion (toxic gastritis and pyloric stenosis), of benzene. Myelofibrosis (a form of aplastic anemia) was reported by a gasoline station attendant who had been exposed to benzene by inhalation, and probably also through dermal contact. Myalgia was also reported in steel plant workers exposed to benzene vapors. Reports of renal effects in humans after benzene exposure consist of kidney congestion after fatal inhalation exposure. Dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapors. Swelling and edema have been reported to occur in a human who swallowed benzene. Studies in animals show systemic effects after inhalation exposure, including cardiovascular effects. Oral administration of benzene to animals has yielded information concerning hepatic effects. A study conducted in rabbits lends support to the finding that benzene is irritating and damaging to the skin and also shows that it is irritating and damaging to the eyes following dermal or ocular application.

Neurological effects have been commonly reported in humans following high-level exposure to benzene. Fatal inhalation exposure has been associated with vascular congestion in the brain. Chronic inhalation exposure has been associated with distal neuropathy, difficulty in sleeping, and memory loss. Oral exposure results in symptoms similar to inhalation exposure. Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain, loss of involuntary reflexes and narcosis, decrease in hind-limb grip strength and tremors, and narcosis, among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behavior. No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals.

Acute inhalation and oral exposures of humans to high concentrations of benzene have caused death. These exposures are also associated with central nervous system depression. Chronic low-level exposures have been associated with peripheral nervous system effects. Abnormalities in motor conduction velocity were noted in four of six pancytopenic individuals occupationally exposed to adhesives containing benzene.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association. Some animal studies provide limited evidence that benzene affects reproductive organs following inhalation exposure. Results from studies of benzene administered orally to rats and mice indicate no adverse effect on male or female reproductive organs at 17 weeks, but at 2 years, endometrial polyps were observed in female rats, preputial gland lesions were observed in male mice, and ovarian lesions were observed in female mice. Results are conflicting or inconclusive as to whether inhalation of benzene vapors reduces the number of live fetuses and/or the incidences of pregnancy. Other studies are negative for effects on reproductive competence.

Epidemiological studies implicating benzene as a developmental toxicant have many limitations, and thus, it is not possible to assess the effect of benzene on the human fetus. Results of inhalation studies conducted in animals are fairly consistent across species and demonstrate that, at levels >47 ppm, benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. Benzene has also been shown to reduce pup body weight in mice. A persistent decrease in the number of erythroid precursors was found in mice exposed in utero. Benzene has not been shown to be teratogenic, but has been shown to be fetotoxic in animals at high concentrations that are maternally toxic.

Cancer. The strongest evidence for the leukemogenic potential of benzene comes from series of cohort mortality studies on workers exposed to benzene in Ohio (the Pliofilm study) and China (the NCI/CAPM study). The Pliofilm study investigated workers exposed to benzene in three rubber hydrochloride ('Pliofilm') manufacturing plants. Mortality from all leukemias was increased but declined after additional years of follow-up, suggesting that the excess risk diminished with time since exposure. Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously. AML accounted for most of the increased leukemia, and the risk of AML increased with increasing cumulative exposure above 200 ppm-years.

The NCI/CAPM study, a collaboration between the National Cancer Institute and the Chinese Academy of Preventive Medicine, evaluated lymphohematopoietic malignancies and other hematologic disorders in 74,828 benzene-exposed workers employed in 672 factories in 12 cities in China. Findings included increased risks for all leukemias, acute nonlymphocytic leukemia (ANLL), and combined ANLL and precursor myelodysplastic syndromes. These risks were increased at average exposure levels of 10–24 ppm and cumulative exposure levels of 40–99 ppm-years, and tended to increase with increasing average and cumulative levels of exposure.

The results of the Pliofilm and NCI/CAPM studies are consistent with epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent. No significant increases in

leukemia or other lymphohematopoietic malignancies were found in chemical industry workers or petroleum industry workers exposed to lower levels of benzene.

Possible associations between occupational exposure to benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma have been suggested. The risk for mortality from NHL increased with increasing level and duration of benzene exposure in the NCI/CAPM study. The significance of this finding is unclear because NHL mortality was not significantly elevated in the cohort overall, concerns regarding the adequacy of the data have been raised, and increases in NHL were not found in other cohort mortality studies or in case-control studies of benzene-exposed workers.

The risk of mortality from multiple myeloma was increased in one of the early assessments of the Pliofilm cohort. The implication of this finding is unclear because the risk declined to non-significant levels in subsequent follow-up studies, and was not supported by the findings of other cohort mortality studies. Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of multiple myeloma. A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents, petroleum, or petroleum products.

Animal studies provide supporting evidence for the carcinogenicity of benzene. Benzene has been shown to be a multiple site carcinogen in rats and mice following inhalation and oral exposure. Tumors that were increased in rats that were exposed to 200 or 300 ppm benzene by inhalation for 4–7 hours/day, 5 days/week for up to 104 weeks included carcinomas of the Zymbal gland and oral cavity. Mice that were exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life developed a variety of tumors, including thymic lymphomas, myelogenous leukemias, and Zymbal gland, ovarian, and lung tumors.

In oral bioassays conducted by the National Toxicology Program, benzene was administered to rats and mice by gavage at dose levels of 25–200 mg/kg/day on 5 days/week for 103 weeks. Tumors that were induced in the rats included Zymbal gland carcinomas and squamous cell papillomas and carcinomas of the oral cavity and skin. In the mice, benzene caused tumors that included malignant lymphomas, Zymbal gland carcinomas, lung alveolar/bronchiolar adenomas and carcinomas, Harderian gland adenomas, preputial gland squamous cell carcinomas, and mammary gland carcinomas. Similar effects occurred in rats exposed to 50–500 mg/kg/day benzene by gavage on 4–5 days/week for up to 104 weeks and observed for life; induced tumors included carcinomas of the Zymbal gland, oral cavity, forestomach, nasal cavity, and skin. Mice that were similarly exposed to 500 mg/kg/day for 52 or 78 weeks developed Zymbal gland carcinomas, mammary carcinomas, and lung adenomas.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most of the dermal studies were inadequate for cancer evaluation. Many dermal carcinogenicity studies of other chemicals used benzene as a vehicle and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. Two studies classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies. Based on human leukemia data, EPA derived a range of inhalation unit risk values of 2.2×10^{-6} – 7.8×10^{-6} ($\mu\text{g}/\text{m}^3$)-1 for benzene. For risks ranging from 1×10^{-4} to 1×10^{-7} , the corresponding air concentrations range from 13.0–45.0 $\mu\text{g}/\text{m}^3$ (4–14 ppb) to 0.013–0.045 $\mu\text{g}/\text{m}^3$ (0.004–0.014 ppb), respectively.

The consensus conclusion that benzene is a human carcinogen is based on sufficient inhalation data in humans supported by animal evidence, including the oral studies in animals. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic (myelocytic) leukemia, whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk range. The oral slope factor ranges from 1.5×10^{-2} to 5.5×10^{-2} ($\text{mg}/\text{kg}/\text{day}$)-1, and for cancer risks from 1×10^{-4} to 1×10^{-7} , the corresponding dose levels are 6.7×10^{-3} – 1.8×10^{-3} to 6.7×10^{-6} – 1.8×10^{-6} $\text{mg}/\text{kg}/\text{day}$, respectively.

Hematological Effects . Both human and animal studies have shown that benzene exerts toxic effects on various parts of the hematological system. All of the major types of blood cells are susceptible (erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A more severe effect occurs when there is hypoplasia of the bone marrow, or hypercellular marrow exhibiting ineffective hematopoiesis so that all types of blood cells are found in reduced numbers. This is known as pancytopenia. A biphasic response (i.e., a hyperplastic effect in addition to destruction of the bone marrow cells) has been observed. Severe damage to the bone marrow involving cellular aplasia is known as aplastic anemia and can occur with prolonged exposure to benzene. This condition can lead to leukemia.

Numerous earlier studies of benzene-exposed workers demonstrated that chronic exposure to benzene air concentrations of 10 ppm or more resulted in adverse hematological effects, which increased in severity with increasing benzene exposure levels. Animal studies support the findings in humans. Significantly reduced counts for all three blood factors (white blood cells [WBCs], red blood cells [RBCs], and platelets); and other evidence of adverse effects on blood-forming units (reduced bone marrow cellularity, bone marrow hyperplasia and hypoplasia, granulocytic hyperplasia, decreased numbers of colony-forming granulopoietic stem cells and erythroid progenitor cells, damaged erythrocytes and erythroblast-forming cells) have been observed in animals at benzene concentrations in the range of 10–300 ppm and above.

Several more recent epidemiological studies have demonstrated hematological effects (including significant reductions in WBC, RBC, and platelet counts) in workers chronically exposed to benzene levels below 10 ppm, and even as low as 1 ppm or less. Results of one of these studies served as the basis for a chronic-duration inhalation MRL for benzene. Other reports demonstrated the lack of clinical signs of hematotoxicity following long-term, low-level occupational exposure to benzene levels below approximately 0.5 ppm (8-hour time-weighted average [TWA]). These investigators utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. The normal range for certain haematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values considered clinically abnormal or adverse may reduce the sensitivity of a particular study to detect meaningful changes at the population level.

Only one study was found that described hematological effects in humans after oral exposure to benzene. No reports describing hematological effects in humans following direct dermal exposure to benzene were found. However, intermediate- and chronic-duration animal studies show that loss of blood elements occurs in animals exposed to benzene in drinking water or by gavage at doses as low as 8–25 mg/kg/day.

Based on information found in the literature, it is reasonable to expect that adverse hematological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to blood elements. Studies show that the hematological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse hematological effects. Deficiencies in various types of blood cells lead to other disorders, such as hemorrhagic conditions from a lack of platelets, susceptibility to infection from the lack of leukocytes, and increased cardiac output from the lack of erythrocytes.

Immunological and Lymphoreticular Effects. Benzene has been shown to have adverse immunological effects in humans following inhalation exposure for intermediate and chronic durations. Adverse immunological effects in animals occur following both inhalation and oral exposure for acute, intermediate, and chronic durations. The effects include damage to both humoral (antibody) and cellular (leukocyte) responses. Human studies of intermediate and chronic duration have shown that benzene causes decreases in the levels of circulating leukocytes in workers at low levels (30 ppm) of exposure and decreases in levels of circulating antibodies in workers exposed to benzene at 3–7 ppm. Other studies have shown decreases in human lymphocytes and other blood elements after exposure; these effects have been seen at occupational exposure levels as low as 1 ppm or less. Animal data support these findings. Both humans and rats have shown increases in leukocyte alkaline phosphatase activity. No studies regarding effects from oral or dermal exposure in humans were located. However, exposure to benzene through ingestion or dermal contact could cause immunological effects similar to those seen after inhalation exposure in humans and inhalation and oral exposure in animals.

Animal studies have also shown that benzene decreases circulating leukocytes and decreases the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies. This has been demonstrated in animals exposed for acute, intermediate, or chronic periods via the inhalation route. This decrease in lymphocyte numbers is reflected in impaired cell-mediated immune functions in mice following intermediate inhalation exposure to 100 ppm of benzene. The impaired cellular immunity after benzene treatment was observed both in vivo and in vitro. Mice exposed to 100 ppm for a total of 100 days were challenged with 104 polyoma virus-induced tumor cells (PYB6). Nine of 10 mice had reduced tumor resistance resulting in the development of lethal tumors. In the same study, lymphocytes were obtained from spleens of benzene-treated mice and tested for their immune capacity in vitro. The results showed that two other immune functions, alloantigen response (capacity to respond to foreign antigens) and cytotoxicity, were also impaired. Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods, and in rats and mice exposed for chronic time periods. A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, the same dose levels at which a decrease in circulating leukocytes was observed. Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours/day for 30 days. The acute-duration inhalation MRL was based on a study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day for 6 days. The intermediate-duration inhalation MRL was based on a study showing delayed splenic lymphocyte reaction to foreign antigens evaluated by in vitro mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day, 5 days/week for a total of 20 exposures.

Based on information found in the literature, it is reasonable to expect that adverse immunological effects might occur in humans after inhalation, oral, or

dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to the immunological system. Studies show that the immunological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites who may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse immunological effects.

Neurological Effects. In humans, results of occupational studies indicate that there is a cause-and-effect relationship between acute inhalation of very high concentrations of benzene and symptoms indicative of central nervous system toxicity. These symptoms, observed following both acute nonlethal and lethal exposures, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. These symptoms are reversible when symptomatic workers are transferred from the problem area. Comparable toxicity in humans has been reported following ingestion of benzene at doses of 125 mg/kg and above. Occupational exposure to benzene has also been reported to produce neurological abnormalities in humans. Electromyographical and motor conduction velocity examinations were conducted on six patients with aplastic anemia, all of whom worked in environments where adhesives containing benzene were used (in one case, air concentrations bracketed around 210 ppm). Abnormalities in motor conduction velocity were noted in four of the six pancytopenic individuals and were thought to result from a direct effect of benzene on the peripheral nerves and/or spinal cord.

In its acute stages, benzene toxicity appears to be due primarily to the direct effects of benzene on the central nervous system, whereas the peripheral nervous system appears to be the target following chronic low-level exposures. In addition, because benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamus-pituitary-adrenal axis. Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive.

Animal studies provide additional support that benzene affects the nervous system following acute inhalation and oral exposures, albeit at extremely high acute exposure levels. Effects reported include narcosis, nervous system depression, tremors, and convulsions. Acute and intermediate inhalation exposures have also been reported to produce adverse neurological effects in animals including a reduction in hind-limb grip strength and evoked electrical activity in the brain, and behavioural disturbances. Effects of benzene on learning were investigated in male hooded rats of the Sprague-Dawley strain given 550 mg/kg of benzene in corn oil or corn oil without benzene, intraperitoneally, on days 9, 11, and 13 postpartum. The rats exposed to benzene exhibited a significantly impaired learning ability when tested on problems of the closed-field, maze-learning task. This sign of neurotoxicity was not observed in control animals. In another study, 47-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4 or 16% crude protein for a 26-day experimental period. Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on days

15–17 of the experimental period. Animals were terminated on day 27. During the experimental period, severe loss of coordination was observed in some rats on the low protein diet immediately after exposure to benzene, but this subsided.

Intermediate oral exposures resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes. Mice exposed to 3 ppm for 2 hours/day for 30 days exhibited increased levels of acetylcholinesterase in the brain. In vitro studies suggest that benzene may have a direct effect on brain cells. Primary astrocyte cultures prepared from neonatal rat cerebella were treated with 3, 6, or 9 mmol/L benzene for 1 hour. ATPase and Mg²⁺-ATPase activity were inhibited in a dose-related manner, and were detected at 78 –92% of control values for ATPase, and 60 –74% of control values for Mg²⁺-ATPase.

These data suggest that humans exposed to benzene in the occupational setting for acute, intermediate, or chronic durations via the inhalation and oral routes are at risk of developing neurological effects. However, benzene levels in ambient air, drinking water, and at hazardous waste sites are lower and not likely to be of concern.