Effects Of Benzene on Human Hematopoiesis

Jorunn Kirkeleit[1], Trond Riise[1], Bjørn Tore Gjertsen[2], Bente E. Moen[1], Magne Bråtveit[1], Øystein Bruserud[2,*]

[1]*Section for Occupational Medicine, Department of Public Health and Primary Health Care, University of Bergen, N-5018, Bergen, Norway;* [2]*Hematology Section, Institute of Medicine, University of Bergen, N-5021 Bergen, Norway*

Abstract: Benzene, an aromatic hydrocarbon that is a natural component of crude oil and natural gas, is toxic to the blood and blood-forming organs. Epidemiological studies have established an association between benzene exposure and acute myeloid leukemia, and increasing evidence also indicates a possible association between benzene and multiple myeloma. A specific benzene-associated myelodysplastic syndrome has also been suggested. Chronic hematotoxic effects of benzene exposure, including reduced lymphocyte, neutrophil and platelet counts in peripheral blood, have been detected at occupational exposure below a level that had previously been considered not to cause any health effects. Whether these abnormalities represent bone marrow damage and/or initial events in the development of a true neoplastic disease is not known. Together with a reported nonlinear relationship between benzene exposure and the level of various metabolites, favoring production of biologically reactive quinones at exposure below 1 part per million, these observations suggest that benzene even at low exposure levels may contribute to the risk of acute myeloid leukemia or myelodysplastic syndrome, especially among genetically susceptible individuals.

1. INTRODUCTION

This review focuses on aspects relevant when individuals with cancer ask their physician about factors in the working environment that might be related to their blood disease and for hematologists seeking updated knowledge on benzene-induced malignancies of the blood and blood-forming organs. Leukemogenesis is a multistep process that is believed to include a combination of mutated signal transduction and perturbed transcription factors [1,2]. The multifactorial origin of most types of cancer, including malignancies of the blood and blood-forming organs, creates difficulty in determining the contribution of single agents. The World Health Organization (WHO) has estimated that benzene, ionizing radiation and ethylene oxide were responsible for 7000 deaths from leukemia in 2000 [3,4]. The estimated fraction attributable to these risk factors was 2% in the WHO study compared with a range of 0.8–2.8% for the United States [5] and 18% for men in Finland [6]. Although the risk associated with occupational exposure is generally several orders of magnitude less than for active smoking, dietary factors and alcohol consumption in the general population, it is high in certain groups of workers.

Benzene, an aromatic hydrocarbon that is a natural component of crude oil and petroleum products, is toxic to the blood and blood-forming organs. The cells of the hematopoietic system are the most sensitive target organs. Repeated occupational benzene exposure over long periods of time may affect several hematopoietic parameters [7-11] and eventually induce malignancies of the blood and blood-forming organs. Benzene exposure has been causally associated with increased risk of acute myeloid leukemia (AML) [12,13], and the associations with multiple myeloma [14,15] and non-Hodgkin's lymphoma [16,17] have been thoroughly debated. No clear evidence indicates any threshold level below which benzene does not cause hematotoxic effects in humans [18], and recent studies indicate that exposure to benzene at levels previously considered not to cause any health effects induce hematotoxicity and an increased risk of malignancies of the blood and blood-forming organs [11,19,20].

*Address correspondence to this author at the Hematology Section, Institute of Medicine, University of Bergen, N-5021 Bergen, Norway;
Tel: +4755972997; Fax: +4755972950;
E-mail: oystein.bruserud@helse-bergen.no

2. BENZENE EXPOSURE

2.1. Occupational Exposure Limits for Benzene

Occupational exposure limits (OEL) are set to protect workers from excessive exposure to toxic chemicals in the workplace. An OEL defines the maximum average concentration of a chemical in the breathing zone acceptable for a normal 8-hour working day for 5 days a week. The OEL is often accompanied by a short-term exposure limit, which is the maximum average concentration to which workers should be exposed for a short period of time (usually 15 minutes). As the hematotoxic and leukemogenic effects have been identified at ever-lower levels, the OEL for benzene has been extensively revised and reduced from 100 parts per million (ppm) in 1946 to values ranging from 0.1 to 1 ppm in 2008 [21]. The American Conference of Governmental Industrial Hygienists set a Treshold Limit Value of 0.5 ppm in 1997, and the European Union has established a legal binding limit value of 1 ppm [22].

2.2. Sources of Benzene Exposure

2.2.1. Occupational Exposure to Benzene

For most job categories the reported full-shift benzene exposure in workers' breathing zone are normally low compared with present OEL. This applies to producing crude oil and natural gas [23-27], refining petroleum products [23,28-

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30], distributing petrol and other petroleum products [23,30-32] and working in car repair shops and petrol stations [33,34].

However, although the mean exposure for long-term sampling during ordinary activity in producing crude oil and natural gas are well below 1 ppm benzene most of the time, exposure levels up to 18 ppm (57.6 mg/m^3) have been reported [27]. Similarly, exposure ranges from below 0.01 to 4.6 ppm in the production of benzene and when refining other petroleum products [28,29,35] and between <0.002 and 32.5 ppm for the distribution of various petroleum products [31,32]. Some specific tasks typically lasting for less than 1 hour, such as tank cleaning and loading of petrol, may cause high short-term exposure [24,27,29,30,36-40].

Workers employed in car repair shops, car recycling, petrol stations and transport are potentially exposed to benzene through their contact with petrol. Reported full-shift exposure ranges between <0.01 and 28.02 ppm [33,34,41,42]. However, the exposure to these groups of workers is probably declining since the benzene content in petrol has been reduced through the implementation of new regulations and recommendations, at least in Europe, United States and Canada [43]. van Wijngaarden & Stewart [44] reviewed the exposure levels in other industries such as chemical manufacture, rubber tire manufacture, steel work, laboratories, waste collection and disposal and firefighting.

2.2.2. Non-Occupationally Related Sources of Benzene

The major sources of benzene exposure for the general population are tobacco smoking and benzene emitted into ambient air during refueling of petrol and the combustion of petrol and other organic materials [45-47].

Smoking. Cigarette smoke is a known source of benzene and its metabolite hydroquinone [48]. Kim *et al.* predicted that smoking 20 cigarettes per day would be equivalent to an occupational exposure of 26 μg/m^3 (approximately 0.008 ppm) [49], resulting in 52% more hydroquinone and 20% more catechol in smoking individuals than observed in non-smoking control subjects [50]. The estimated benzene exposure was in accordance with previous predictions for an urban smoker consuming 20 cigarettes per day [46]. In contrast, in an experimental study smokers not occupationally exposed to benzene reached a morning concentration of benzene in blood of up to 13 nmol/l after smoking four or five cigarettes [51], which is estimated to be equivalent to benzene exposure in the breathing zone of as much as 0.3 ppm averaged over an 8-hour shift [52].

Ambient air. For the general population, the European Union has established a limit value of benzene in ambient air of 5 μg/m^3 (approximately 0.0016 ppm) averaged over a calendar year [53]. In the United Kingdom, annual mean concentrations at urban sites range from 2.2 to 8.0 μg/m^3, and data from rural sites showed a mean annual concentration of 1.3 μg/m^3 [46]. The maximum hourly concentrations measured in urban and rural sites were 139 and 15.4 μg/m^3, respectively. Similar results have been reported elsewhere [45,47,54]. Hence, the exposure level posed on the general population through refueling and combustion of petrol, passive tobacco smoking, and point sources such as petrochemical plants or oil refineries is considerable lower than the level experienced by the benzene-exposed worker.

3. TOXICOKINETICS OF BENZENE

3.1. Absorption

Inhalation is the most important route of absorption during occupational exposure to benzene. Humans absorb 30–52% of inhaled benzene, depending on the benzene concentration, length of exposure and pulmonary ventilation [51,55,56]. Benzene penetrates skin [57-59]. However, dermal absorption of benzene is not extensive, as it evaporates quickly due to high vapor pressure. Hence, under normal working conditions, dermal absorption of benzene is probably of minor importance [59-62].

3.2. Metabolism

The liver is the major site of metabolism of benzene [63]. Benzene is detoxified in two phases. During phase I, benzene is oxidized by cytochrome P450 2E1, forming benzene oxide, an electrophilic reactive intermediate. Subsequently, benzene oxide is metabolized by three pathways [63]:

1) rearrangement non-enzymatically to form phenol;
2) hydration by epoxide hydrolase to 1,2-benzene dihydrodiol, which in turn can be oxidized by dihydrodiol dehydrogenase to form catechol; and
3) glutathione conjugation with glutathione *S*-transferase to form a premercapturic acid, which is converted to phenylmercapturic acid.

Phenol can undergo subsequent hydroxylation to hydroquinone, with the consecutive production of *p*-benzoquinone and 1,2,4-trihydroxybenzene. Alternatively, phenol can be hydroxylated to catechol, which is converted to *o*-benzoquinone. The benzene ring can also be opened either at the benzene oxide or oxepin stage, forming muconaldehyde. All these metabolites can then undergo a phase II metabolism, leading to excretion of glucuronide and sulfate conjugates, mercapturic acid ring-opened metabolites and DNA adducts in urine [63].

3.2.1. Production of Toxic Metabolites in the Target Organ

Benzene itself is not regarded as a toxic substance. Benzene toxicity is believed to involve biological interactions of multiple reactive benzene intermediates with multiple cellular targets within the bone marrow. Especially hydroquinone, *p*-benzoquinone, catechol and muconaldehyde, alone or in combination, are reported to be the most potent metabolites in producing hematotoxicity [63,64].

Beside the enzyme CYP 2E1 [65], the bone marrow contains several peroxidases; the most prevalent is myeloperoxidase [66-68]. Phenol, catechol and hydroquinone are transported to the bone marrow, where myeloperoxidase is responsible for converting these metabolites to several biologically reactive quinones [68].

3.2.2. Nonlinear Benzene Metabolism

The production of the major benzene metabolites [49,50], as well as albumin adducts of benzene oxide and benzoquinones [69,70], exhibit a nonlinear dose-response relationship attributable to saturated metabolism of benzene. For the *S*-phenylmercapturic acid there was an increasing production along with increasing benzene exposure. However, for all major metabolites competing for the cytochrome P450 2E1

system, such as phenol, catechol, hydroquinone and muconic acid, there was in fact a decreasing trend after a transition level around 0.03 ppm [49,50]. Above this level the production of catechol and phenol dropped by 4.4 and 16-fold already when reaching exposure of 0.27 ppm, while the reduction for hydroquinone and muconic acid was only marginal. Hence, at low doses (below 1 ppm) the metabolism favored the production of hydroquinone and muconic acid. Hydroquinone is the precursor of the toxic 1,4-benzoquinone, whereas muconic acid is derived from the extremely reactive and toxic muconaldehydes. From these results, it was concluded that workers exposed to benzene below 0.1 ppm metabolize benzene about nine times more efficiently and therefore more adversely than do heavily exposed workers.

4. BENZENE TOXICITY IN HUMANS

4.1. Hematotoxicity Caused by Chronic Benzene Exposure – Bone Marrow Damage or Leukemogenesis?

Several previous studies [7,8,10,11,71,72] have described abnormalities in myeloid and lymphoid cells among workers with long-term exposure to benzene. These abnormalities have been observed even after low benzene exposure (<1 ppm) and include decreased circulating white blood cells, $CD4^+$ T cells, $CD4^+/CD8^+$ ratio and B cells, neutrophils and platelets [11]. Tests for linear trends using the benzene concentration in air as a continuous variable were significant for platelets and various leukocyte subsets except monocytes and $CD8^+$ T cells. Diminished thymus function did not appear to mediate the lymphotoxicity of benzene [73]. Benzene affected progenitor cell colony formation significantly more strongly than the number of mature blood cells. The genotype of benzene detoxifying and activating enzymes influenced leukocyte toxicity – in particular myeloperoxidase and NAD(P)H:quinone oxireductase, showing a strong gene-dosage effect. Taken together, these results suggest that long-term exposure to benzene, even below 1 ppm, can induce hematotoxicity. However, whether these reduced levels in circulating blood cells simply represent bone marrow damage or the initial steps of a neoplastic bone marrow disease cannot be determined.

Table **1** presents studies assessing outcomes on the blood and blood-forming organs in benzene-exposed workers. Since the studies have reported effects on the blood and blood-forming organs according to different metrics of exposure, only the directions of the altered level in the respective studies are given as arrows. However, two of the studies assessed the reduction in several exposure groups. Lan *et al.* [11] reported that the reductions in the various exposure groups compared with the controls were 14.5–26.4% for white blood cells, 8.0–15.5% for total lymphocytes, 18.2–32.1% for granulocytes and 7.0–25.2% for platelets. The corresponding ranges reported from Qu *et al.* [10] were 13.0–15.6% for red blood cells, 4.3–29.1% for white blood cells and 15.7–38.1% for neutrophils. For both these studies a significant dose-response relation were found. Importantly, several studies [74-76] also reported no decrease in blood cell counts among benzene-exposed workers or that some of the hematological parameters previously reported to be sensitive to benzene exposure, such as total number of white blood cells, neutrophils, eosinophils and monocytes, were in fact significantly increased in the exposed group compared with controls [77]. The differences in the findings of these studies could be related to the reported lower mean exposure and the use of routinely collected health surveillance data in the negative studies. Further, more of the positive studies have been done on Asians, shown to have a higher risk of benzene toxicity than other ethnic groups due to genetic polymorphism in some enzymes involved in metabolizing benzene [78,79]. Nevertheless, overall these studies show that benzene induces a hematotoxic effect in both myeloid and lymphoid cell lines.

Studies have also reported that benzene exposure affects the proteins of the immune system. These effects include reduced serum immunoglobulins [72,80-82], an anti-benzene antibody response [83] and reduced complement levels [84].

4.1.1. Benzene-Associated Hematotoxicity and Growth Factor Signaling

Benzene-exposed workers had reduced expression of various cytokines, including the CXC-chemokines CXCL4 (platelet factor 4) and connective tissue–activating peptide (CTAP-III), compared with unexposed workers [85]. These chemokines are mainly released by platelets, but the levels showed no correlation with peripheral blood platelet counts. Thus it was concluded that, the altered levels of these mediators probably reflect a qualitative difference between thrombocytes derived from benzene-exposed and -unexposed individuals. Twenty-nine genes, including the two chemokines CXCL4 (downregulated) and chemokine (C-X-C motif) ligand 16 (upregulated), were likely to be differentially expressed in workers heavily exposed to benzene (mean exposure = 44 ppm) compared with unexposed workers [86]. Thus, alteration in the cytokine network, and especially the chemokine system, seems to be important in benzene toxicity.

4.2. Benzene-Associated Aplastic Anemia

Chronic exposure to high benzene concentrations has long been associated with aplastic anemia [87]. Most of these cases have been diagnosed based on pancytopenia in peripheral blood, and direct examination of the bone marrow is missing for most cases [for references see 88]. Given the recent observation of hypoplastic myelodysplastic syndrome in benzene-exposed individuals (see below) [88], the reported association between benzene exposure and aplastic anemia might at least partly represent an association between benzene exposure and myelodysplasia.

4.3. Benzene-Associated Myelodysplastic Syndrome

Several studies [88-90] have described an association between benzene exposure and myelodysplastic syndrome, with Irons *et al.* [88] reporting a unique form of benzene-associated myelodysplasia. Irons *et al.* included a relatively low number of patients, but they underwent detailed examination. Patients were referred to hospitals based on initial clinical presentation and/or a medical history of occupationally related benzene intoxication. Their benzene exposure was independently verified, and estimated full-shift exposure averaged between 50 and 300 ppm, which is very high compared with the OEL of 1 ppm benzene or less in most western countries [21]. The patients had been exposed for varying periods of time ranging from 6 to 22 years and were removed from exposure on average 2.7 years before evalua-

Table 1. Selected Studies on Outcomes on the Blood and Blood-Forming Organs Among Benzene-Exposed Workers

Study Population and Design	Benzene (ppm) Measure of Central Tendency (Range)	Outcome on the Blood and Blood-Forming Organs						Reference
		Red Blood Cells	White Blood Cells				Platelets	
			Total	Lymphocytes	Neutrophils	Granulocytes		
Benzene-exposed workers – USA Routinely collected surveillance data	Range of geometric means: 0.01–1.40 ppm	→	→	→	NA	NA	→	[74]
Benzene-exposed workers – USA Routinely collected surveillance data	Mean: 0.55 ppm (0.01–87.7)	→	→	→	NA	NA	→	[75]
Benzene-exposed workers – China Cross-sectional study	Median: 31 ppm (1–328)	↓	↓	↓	NA	NA	↓	[7]
Production of natural rubber film – USA Matched case-control design	Maximum daily dose estimate: 34 ppm	↓	↓	NA	NA	NA	NA	[8]
Petroleum workers – USA Routinely collected surveillance data	Mean: 0.81 ppm (0.14–2.08)	↓	→	NA	NA	NA	↓	[71]
Shoemaking-industry – Croatia Cross-sectional study	Median: 5.9 ppm (1.9–14.8)	NA	NA	↓	NA	NA	NA	[72]
Benzene-exposed workers – China Cross-sectional study	Median: 3.2 ppm (0.06–122)	↓	↓	(↓)	↓	(↓)	→	[10]
Shoe-making industry – China Cross-sectional study	Means (ppm): <1, 1 to <10 and ≥10 ppm	NA	↓	↓	NA	↓	↓	[11]
Petrochemical workers – USA Routinely collected surveillance data	Means: 1977–1987: 0.6 ppm 1988–2002: 0.14 ppm	→	→	→	NA	NA	→	[76]

→: no difference between exposed workers and reference group. ↓: significantly reduced level in exposed workers compared with reference group. ↑: significantly increased level in exposed workers compared with reference group. NA: the parameter was not assessed or reported.

tion. Thus, these observations are probably not representative for western industry, where the time-weighted average exposure is generally much lower and the high exposure during specific tasks usually lasts for brief periods of the work shift [21,24,26].

Table **2** summarizes the characteristics of benzene-associated myelodysplastic syndrome from workers in China with long-term exposure [88]. A striking feature is the bone marrow hypocellularity observed in 17 of the 23 patients. For many cases, there was a lack of concordance between the severity of the marrow abnormalities and peripheral blood cytopenia. Another striking characteristic was the high frequency of normal cytogenetics, differing from chemotherapy-induced myelodysplastic syndrome that is characterized by certain chromosomal deletions (alkylating agents) or translocations (topoisomerase inhibitors) [91]. Finally, the presence of immune system abnormalities is not surprising. Myelodysplastic syndrome is associated with immune system abnormalities, reflected in the polyclonal hypergammaglobulins detected in about one third of patients [92]. A minority of myelodysplastic syndrome patients also develop autoimmune disease [92,93].

4.4. Genetic and Epigenetic Effects in Benzene-Exposed Individuals

4.4.1. Chromosomal Abnormalities in Benzene-Exposed Individuals

Monosomy of chromosome 7, trisomy 8 and translocations between chromosomes 8 and 21 (t(8;21)) are chromosomal changes observed in AML [94,95]. An increased inci-

Table 2. Clinical and Biological Characteristics of Myelodysplastic Syndrome Developing After Long-Term Exposure to High Benzene Concentrations; A Summary of Reported Observations for 23 Chinese Workers Previously Exposed to Benzene [88]

Patient characteristics
Seven men and 16 women, mean age 44.4 years (standard deviation = 7.8)
Median duration of exposure 14 years, range 6–22 years
Median time since last exposure 36 months, range 0–95 months Estimated full-shift exposure averaging between 50 and 300 ppm benzene
Bone marrow morphology
Hypocellularity (17 of 23) with uneven distribution of remaining hematopoietic cells throughout the marrow
Dyserythropoiesis with macrocytic and megaloblastic changes, myeloid cells with megaloblastic alterations and abnormal cytoplasmic morphology
Prominence of abnormal eosinophilic precursors (22 of 23)
Hematophagocytosis (16 of 23)
Genetic abnormalities
Normal cytogenetics in all patients
No Flt3 mutations detected
Immunological abnormalities
Increased levels of circulating large granular lymphocytes
Decreased $CD4^+/CD8^+$ T-cell ratio in the bone marrow
Clonal or oligoclonal proliferation of bone marrow T lymphocytes determined by analysis of clonal rearrangements of T-cell receptor chains (14 of 23)

dence of these aberrations has been reported in workers exposed to benzene [96]. Studies of chromosomal abnormalities in blood cells have suggested that benzene metabolites particularly produce monosomy of chromosomes 5 and 7 in human lymphocytes from healthy workers exposed to benzene [97] and in human bone marrow cells obtained from healthy volunteers [98,99], with bone marrow cells being more susceptible than lymphocytes. The aberrations t(8;21) and trisomy 8 have also been reported [100]. In a recent pilot study comparing six benzene-exposed workers with five unexposed referents, Zhang *et al.* [101] reported that benzene exposure was associated with monosomy of chromosomes 5, 6, 7 and 10 and with trisomy for chromosomes 8, 9, 17, 21 and 22.

A dose-dependent induction of long-arm deletion of chromosome 6 [del(6q)] and an increased frequency of translocation t(14;18) among highly exposed workers have been reported [102]. Both t(14;18) and del(6q) are also frequently observed in lymphoma patients [103-105]. Induction of t(4;11) and t(6;11), common in therapy-related leukemia due to topoisomerase II–inhibiting drugs, was not found. Taken together, these observations suggest that benzene produces selective effects on certain chromosomes. Another study described an association between chromosomal abnormalities in lymphocytes and the frequency of activated T cells in peripheral blood among workers exposed to benzene, styrene, polycyclic aromatic hydrocarbons and/or solvents and unexposed referents [106], which suggests a link between genotoxicity and immunomodulation.

An important question then is why the benzene-associated monosomies and trisomies are not detected in the patients with benzene-associated myelodysplastic syndromes reported by Irons *et al.* [88] (Table **2**). The present scientific literature cannot answer this question, but possible explanations are: (i) variation in exposure; (ii) certain abnormalities may predispose to the direct development of leukemia without preleukemic myelodysplasia; or (iii) cells with these abnormalities may not survive long enough to form the basis for disease development.

4.4.2. Oxidative stress, DNA damage and changes in DNA methylation patterns

Several studies have reported oxidative stress [107,108] and increased single-strand breaks in the DNA of circulating blood cells [108-110] among workers exposed to benzene. A study of filling station attendants exposed to benzene [107] found a significant association between benzene exposure and the urinary oxidative DNA adduct 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative stress [111]. Liu *et al.* [112] reported that both concentrations of benzene in air and urinary *t*,*t*-muconic acid were significantly associated with 8-OHdG in lymphocyte DNA, together with a correlation between 8-OHdG and micronucleus frequency. Workers exposed to gasoline, with an average benzene exposure of 0.13 ppm over a full shift, had an increase in single-strand breaks in DNA of leukocytes compared with unexposed controls. Urinary 8-OHdG increased over the shift

among exposed workers, and the increase was significantly associated with the exposure to benzene during the shift.

In another study, comet assays were carried out to evaluate DNA damage (single-strand breaks) in T and B lymphocytes and granulocytes from benzene-exposed workers [109]. Significantly higher DNA damage, measured as tail moments, was reported among exposed workers than among referents. B lymphocytes, which have the shortest life span, were more sensitive to low levels of benzene than were the T lymphocytes and granulocytes. Similar results have been reported for benzene-exposed workers from a range of industries with a mean benzene exposure of 0.27 ppm (range 0.005–2.03 ppm) [110].

Bollati *et al.* [113] studied aberrant DNA methylation patterns in gas station attendants and traffic police officers exposed to low benzene levels. Benzene exposure was associated with a significant reduction in global methylation and gene-specific hypermethylation (*p15* gene) and hypomethylation (*MAGE-1* gene). Loss of imprinting was only found in exposed subjects, but no dose response was found. Similar aberrant DNA methylation patterns have been found in subjects with AML [114].

4.5 Possible clues to the Mechanisms Behind the Effect of Benzene on Hematopoiesis

4.5.1. Genotoxic Effects of Benzene

Benzene's metabolites are non-mutagenic or weak mutagens [115,116]. In contrast to most other carcinogens, benzene is not assumed to directly damage the DNA. The mechanisms behind the genotoxic effect of benzene's metabolites are proposed to include concerted action with the generation of active oxygen species via redox cycling, adduct formation and damage to DNA-associated proteins such as topoisomerase II and the mitotic apparatus, with consequent damage including DNA strand breakage, mitotic recombination, chromosome translocations and aneuploidy [115,116].

Topoisomerases are nuclear enzymes that play important roles in maintaining the integrity of the genome during replication, recombination and the separation of sister chromatids [117]. Experimentally, the benzene metabolite hydroquinone can be activated to a topoisomerase II inhibitor by myeloperoxidase and H_2O_2 [118]. Benzene-derived quinones also enhance DNA cleavage and inhibit DNA ligation mediated by topoisomerase IIα [119]. However, human studies provide little evidence that inhibition of topoisomerase II plays a role in benzene's leukemogenic effects.

Benzene induces gene-duplicating mutations in exposed humans [120]. One of the most frequent mutations in AML is a duplicating mutation of the receptor tyrosine kinase Flt3 [121], but a cause–effect relationship between this mutation and benzene exposure has never been reported.

Alternative mechanisms, involving oncogene activation such as c-Myb [122] and aryl hydrocarbon receptor activation [123,124], have been proposed to be involved in benzene-induced hematotoxicity. Aryl hydrocarbon receptor activation by benzene metabolites suggests biological effects of benzene at low doses. Together, these studies suggest a complex mechanism of benzene-induced malignancies, including genotoxic damage, DNA repair failures and altered oncogenic signaling.

4.5.2. Benzene-Induced Dysfunction of Cell Cycle Regulation

Through the presence of myeloperoxidase [66,68], an enzyme involved in forming the active benzene metabolite hydroquinone [67], the bone marrow may be particular prone to benzene-induced toxicity. Hydroquinone affects the differentiation of myeloblasts in mice and myeloid-derived cell lines [125] and may represent a mechanism for acute and chronic toxicity. Several experimental animal and *in vitro* studies [126-130] have reported benzene-induced dysregulation of cell cycle regulation in hematopoietic progenitor cells, the cells reported to be most sensitive to benzene's toxic effects.

Normal function of the tumor suppressor protein p53 is essential in DNA repair, cell cycle control and cell apoptosis. Yoon *et al.* [126] reported that benzene suppresses the cell cycle in hematopoietic progenitor cells (colony-forming unit–granulocyte monocyte progenitor) in mice by p53-mediated overexpression of p21, a cyclin-dependent kinase inhibitor. This resulted not simply in suppression of hematopoiesis but rather in a dynamic change of hematopoiesis during and after benzene exposure (oscillatory changes), possibly through the genetic and epigenetic effects of benzene [127]. It has also been reported that bone marrow cells in p53-deficient mice expressed significantly reduced levels of many key genes involved in the p53-regulated DNA damage response pathways (*p21*, *gadd45*, *cyclin G*, *bax* and *bcl-2*) after chronic exposure to benzene [128]. In human CD34 cells treated with the benzene metabolite 1,4-benzoquinone, induction of the cyclin-dependent kinase inhibitor p21 at the mRNA level was found, while no changes in mRNA expression were observed for p53 or the DNA repair genes *rad51*, *xpc*, *xpa*, *ku80* and *ape1* [131].

4.5.3. Effects of Benzene Metabolites on the Production of Chemokines and Cytokines

Gillis *et al.* [132] investigated the effects of exposure to benzene metabolites on the immune system measured by the secretion of extracellular cytokines by human peripheral blood mononuclear cells exposed to benzene metabolites. Hydroquinone enhances cytokine-dependent clonal proliferation of a subpopulation of human $CD34^+$ BM cells, which appears to be mediated via the extracellular signal-regulated kinase/activation protein-1 signaling pathway [133-136]. Hydroquinone has also been reported to synergize with tumor necrosis factor α to induce apoptosis in human $CD34^+$ hematopoietic progenitor cells by inhibiting nuclear factor-kappa B [137], and to inhibit macrophage-mediated immune responses by modulating intracellular signaling and protective mechanisms [138].

5. EPIDEMIOLOGICAL STUDIES OF BENZENE TOXICITY

5.1. Leukemia

Epidemiological studies of leukemia provide strong evidence for a causal association between exposure to benzene and leukemia [12]. Numerous mortality and incidence studies assessing this association have been performed mainly in benzene-exposed workers from the production of natural

rubber film [139-142], shoe-producing industry [143,144] and the petroleum industry [19,20,145-157], but other industries and occupations have also been examined [90,158-160]. Table **3** provides an overview of studies that includes estimates of the quantitative risk of benzene exposure.

The association between benzene exposure and leukemia is strongest for AML and less clear for the other subtypes [12,13]. Several studies on mortality rates or cancer incidence assessing the risk of leukemia in benzene-exposed cohorts have reported an increased risk of AML [19,20,90,142,146,147]. A recent review on benzene exposure and leukemia subtypes including nine cohorts and 13 case–control studies from several industries [13] showed a high and significant risk of AML, with a positive dose–response relationship across study designs. A study of a cohort of offshore workers in the petroleum industry published after this review [20] showed a relative risk of 2.9 among workers assumed to have the most extensive contact with crude oil.

There is a biologically plausible basis for suggesting benzene as a causal factor for acute lymphoblastic leukemia and chronic myeloid leukemia, which also develop in the bone marrow, and some studies of benzene-exposed workers have reported such an increased risk. The assessment of the risk of these malignancies is mainly hampered by their rarity, and Schnatter *et al.* [13] concluded that the data for these leukemia subtypes were sparse and inconclusive. An older meta-analysis of leukemia subtypes including 19 studies of various populations of petroleum workers [148] found no excess of acute lymphoblastic leukemia and chronic myeloid leukemia. However, the power of these analyses was low, as indicated by the failure of the same meta-analyses to show a significantly increased risk for AML.

Some epidemiological studies [152,157] have reported an association between benzene exposure and chronic lymphocytic leukemia. The main problem in assessing the risk of chronic lymphocytic leukemia is the different disease classifications used over time [161] and the lack of specific information on chronic lymphocytic leukemia in most studies [162]. Schnatter *et al.* [13] concluded that the risk of developing chronic lymphocytic leukemia was only increased in some case–control studies, but not in the cohort studies, arguing against a major effect.

5.1.1. Temporal Variation in Risk and Reported Latency Between Exposure and the Development of Leukemia

Several authors have discussed the temporal variation of the risk of developing leukemia after exposure to benzene. For the Pliofilm cohort [140,141], the increased risk was reported to be attributable primarily to exposure occurring during the last 10 years preceding death [163,164], and the risk was highest in the first years after exposure ended [164]. Glass *et al.* [165] reported a similar pattern before leukemia was diagnosed in the Health Watch cohort from the Australian petroleum industry. Further, in the large cohort of benzene-exposed workers in China, the risk for the combination of AML and related myelodysplastic syndromes was highest among workers who had only recent exposure (<10 years prior to diagnosis) [90].

Although the time estimates reported in studies on benzene-induced malignancies of the blood and blood-forming organs represent a combination of latency and the effect of cumulative exposure and period of employment, the observations are compatible with a wide range of latency periods for AML induced by benzene. Latency periods for leukemia, representing years between first exposure and death, ranged from 2 to 51 years in the Pliofilm cohort [141]. Among offshore workers exposed to benzene during the production of crude oil, the median time between the first year of registered engagement and the diagnosis of AML was 6 years (range 5–21) [20]. The median latency time for lymphomas and leukemias combined was 9.5 years among PhD fellows at a university laboratory and 7.5 years for students attending a basic organic chemistry course where benzene was used [166]. On the other hand, several mortality studies of workers from the petroleum industry have shown an increased risk of leukemia along with increasing duration of employment, with the highest risk among workers employed for more than 20–30 years [146,147,150,157]. The information given by Costantini *et al.* [144] enabled the calculation of mean and median times from first exposure to death from lymphohematopoietic cancer of 28.4 and 31.5 years, respectively (range 3-49 years).

5.2. Multiple Myeloma

The association between exposure to benzene and multiple myeloma is contentious [14,15,167,168]. The conclusion in an older meta-analysis of 22 cohort mortality studies in the petroleum industry was that these workers were not at any increased risk of developing multiple myeloma [167]. In contrast, a more recent meta-analysis including seven cohort studies focusing on benzene-exposed workers found a significant excess in the relative risk (RR = 2.13) [168].

Most of the studies included in the negative meta-analysis [167] were performed on a cohort known to be exposed to relatively low concentrations of benzene. In addition, multiple myeloma probably has a longer latency period than AML [20,139,140,159], making the causal relation between benzene and multiple myeloma more difficult to detect epidemiologically. It has therefore been claimed that asking whether benzene causes multiple myeloma is an unreasonable question in a cohort in which the benzene effect is too weak to even observe an increased risk of AML [15]. Further, a marked limitation of the studies in the petroleum industry is the likely presence of a healthy worker effect, with overall mortality and overall cancer incidence among these workers significantly lower than in the general population [153,154,156,169]. The healthy worker effect might mask increased risks of multiple myeloma even in studies capable of showing an increased risk of AML.

Interestingly, in the cohort described by Sathiakumar *et al.* [147], with an increased risk of AML (RR = 1.6) among men ever employed in the oil and gas sector, unpublished results also showed a borderline significantly increased risk of multiple myeloma (RR = 2.9) [170]. Sailors exposed to cargo vapor from gasoline and other light petroleum products on tankers had an increased risk of multiple myeloma [171]. The recent finding of an increased risk of multiple myeloma (RR = 2.49) among upstream petroleum workers also showing an increased risk of AML (RR = 2.89) provides further evidence of an association between benzene exposure and the risk of multiple myeloma [20]. Several studies have

Table 3. Overview of Studies Assessing the Risk of Leukemia and/or Acute Myeloid Leukemia in Cohorts Occupationally Exposed to Benzene that Also Include Risk Estimates for Cumulative Benzene Exposure (ppm-years). Risk Estimates Given in Bold are Statistically Significant

Industry	Design	*n*	Exposure Metric	Exposure	Leukemia	Acute Myeloid Leukemia	Acute Nonlymphocytic Leukemia or Acute Leukemia	Ref.
Manufacture of natural rubber	Cohort study (mortality) – USA	1165	All exposed		SMR **3.4**	—	—	[140]
			Cumulative exposure	0.001–40 ppm-years 40–200 ppm-years 200–400 ppm-years >400 ppm-years	SMR 1.1 SMR 3.2 SMR **11.9** SMR **66.4**	—	—	
	Cohort study (mortality) – USA Update of Rinsky et al. 1987	1291	All exposed		SMR **2.5**	—	—	[141]
			Cumulative exposure	0.001–39.99 ppm-years 40–199.99 ppm-years 200–399.99 ppm-years >400 ppm-years	SMR 1.5 SMR 3.2 SMR 5.6 SMR **24.0**	—	—	
	Cohort study (mortality) – USA Update of Rinsky et al. 1987	Not given	All exposed		—	SMR **5.0**	—	[142]
			Cumulative exposure	<40 ppm-years 40–200 ppm-years 200–400 ppm-years >400 ppm-years	—	SMR 1.2 SMR — SMR **27.2** SMR **98.4**	—	
Variety of industries and occupations using benzene	Cohort study (mortality) – China	74,828	All exposed		RR **2.5**	—	RR **4.1**	[90]
			Cumulative exposure	<40 ppm-years 40–99 ppm-years ≥100 ppm-years	RR 1.9 RR **3.1** RR **2.7**	—	RR 2.7 RR **6.0** RR **4.4**	
Manufacture of shoes	Cohort study (mortality) – Italy	1687	Cumulative exposure	<40 ppm-years 40–99 ppm-years 100–199 ppm-years ≥200 ppm-years	SMR 1.3 SMR 4.1 SMR 2.5 SMR **5.1**	—	—	[144]
Petroleum industry	Nested case-control study – Australia	33 cases of leukemia	Cumulative exposure	≤1 ppm-years > 1–2 ppm-years > 2–4 ppm-years > 4–8 ppm-years >8–16 ppm-years > 16 ppm-years	OR 1.0 OR 3.9 OR **6.1** OR 2.4 OR **5.9** OR **98.2**	—	—	[19]
				≤4 ppm-years >4–8 ppm-years >8 ppm-years	—	—	1.0 OR 0.5 OR **7.2**	
Petroleum industry	Nested case-control study – United Kingdom	91 cases of leukemia	All exposed		OR 1.0	OR 1.0	—	[150]
			Cumulative exposure	<0.45 ppm-years 0.45–4.49 ppm-years 4.5–44.9 ppm-years ≥45 ppm-years	OR 1.0 OR 1.4 OR 2.5 OR 1.4	OR 1.0 OR 2.2 OR 2.8 OR —	—	

Table 3. contd….

Industry	Design	*n*	Exposure Metric	Exposure	Leukemia	Acute Myeloid Leukemia	Acute Nonlymphocytic Leukemia or Acute Leukemia	Ref.
Petroleum industry – distribution	Nested case-control study – Canada	14 cases of leukemia	Cumulative exposure	<0.45 ppm-years >0.45–4.5 ppm-years >4.5–45 ppm-years >45 ppm-years	OR 1.0 OR 0.4 OR 0.2 OR 1.5	—	—	[149]
Chemical plants	Cohort study (mortality) – USA	2266	All exposed		SMR 1.1	—	SMR 1.1	[160]
			Cumulative exposure	<28.3 ppm-years 28.3–79.1 ppm-years >79.1 ppm-years	SMR 0.6 SMR 2.0 SMR 2.2	—	SMR 0.9 SMR 1.5 SMR 1.6	
Chemical plant	Cohort study (mortality) USA	4417	All exposed		SMR 1.3	—		[159]
			Cumulative exposure	No exposure <1 ppm-years 1–6 ppm-years >6 ppm-years	SMR 1.0 SMR 0.7 SMR 1.4 SMR 1.7	—	SMR 0.8 SMR 1.4 SMR 2.7 SMR 2.2	
Gas and electricity utility	Nested case-control study – France	72 cases of leukemia	Cumulative exposure	0 ppm-years 0.1–1.0 ppm-years 1.1–5.4 ppm-years 5.5–16.7 ppm-years ≥16.8 ppm-years	OR 1.0 OR 0.7 OR 1.4 OR 1.9 OR **3.6**	—	OR 1.0 OR 0.3 OR 0.3 OR 1.2 OR **4.6**	[158]
				Never exposed 0.1–5.4 ppm-years ≥5.5 ppm-years	—	OR 1.0 OR 0.2 OR 2.4	—	

SMR: standardized mortality ratio. RR: rate ratio. OR: odds ratio.

also reported that proximity to oil or gas fields represent an increased population risk of developing lymphohematopoietic cancers, including multiple myeloma [172,173]. On the other hand, the increased risk of multiple myeloma reported in petroleum-related cohorts might also be related to exposure to compounds in diesel or engine exhaust other than benzene [174-176].

5.3. Lymphoma

Lymphoma is a group of heterogeneous malignancies. The causes of lymphoma are still largely unknown, but given the potential for benzene to affect the immune system, an association between benzene exposure and non-Hodgkin's lymphoma has been suggested. In a large cohort of workers in China with 10 or more years of benzene exposure, Hayes *et al.* [90] reported an RR of non-Hodgkin lymphoma of 4.2 (95% confidence interval (CI) 1.1–15.9) versus 0.7 (95% CI 0.1–7.2) for workers exposed to benzene for less than 5 years. The increased risk was found only for workers with an average exposure of ≥25 ppm benzene (RR = 4.7). Several other studies have reported an increased risk of non-Hodgkin lymphoma among workers occupationally exposed to benzene [157,177-179] or to a mixture of organic solvents rather than benzene alone [177,180,181].

Except for the unpublished results of a significantly increased risk of non-Hodgkin lymphoma (RR = 2.4) among men ever employed in the oil and gas sector [170] and in petroleum workers hired prior to 1950 (standardized mortality ratio 1.57) [157], most cohorts of petroleum workers with a potential for benzene exposure have reported negative findings on benzene-induced non-Hodgkin lymphoma. However, negative findings in cohorts of petroleum workers might be explained by exposure in this industry not being sufficiently high to induce non-Hodgkin lymphoma or the follow-up time to detect an increased risk of non-Hodgkin lymphoma being too short.

5.4. What Level of Benzene Exposure May Induce Malignancies of the Blood and Blood-Forming Organs?

There is no clear evidence of a threshold level below which benzene does not affect human hematopoiesis, and an increasing number of studies indicate an increased risk of leukemia at levels well below 10 ppm [19,20,90]. However, a marked limitation of studies on benzene-induced malignancies of the blood and blood-forming organs is the lack of good exposure estimates. Table **3** provides an overview of studies that estimate the quantitative risk of benzene exposure. Most studies have focused on describing the average airborne benzene exposure over a full work shift cumulated

over the duration of employment, such as ppm-years, where cumulative exposure levels of 40 and 200 ppm-years represent 40 years of 1 and 5 ppm benzene, respectively. However, some studies are also estimating the risk for other exposure metrics, such as average exposure, exposure intensity and duration of employment.

Which exposure metric best predicts risk? The exposure metric that best predicts the risk of benzene-induced malignancies of the blood and blood-forming organs is not known, and future epidemiological studies need to better describe the variability of occupational benzene exposure. Although some of the studies assessing benzene-induced leukemia in occupational cohorts also include various exposure metrics, such as duration of exposure, exposure intensity and average exposure, most studies focus on describing the average airborne benzene exposure over a full work shift cumulated over the duration of employment, such as ppm-years. However, the number of peak exposures to benzene (above 100 ppm), rather than cumulative exposure, has been proposed to best predict the risk of malignancies of the blood and blood-forming organs [159]. Among petroleum workers, exposure to concentrated benzene has resulted in a higher risk of leukemia than exposure to the same amount of benzene encountered in a more dilute form such as in petrol [19].

Nonlinear metabolism. Emerging knowledge of a nonlinear dose-related production of major metabolites, favoring the production of the hematotoxic quinones at low benzene exposure, implies that previous risk assessments probably underestimated the risk at low exposure. As several researchers [49,50,69] argue, the workers exposed to benzene below 1 ppm will be subjected to the maximum possible mass of metabolites per unit of benzene exposure due to more effective metabolism at low exposure. Further, due to this saturated metabolism, high concentrations of benzene, both as a time-weighted average and as transient peak exposures, might have diminished the effect on workers' risk of developing hematotoxic effects or malignancies of the blood and blood-forming organs as compared with low benzene exposure.

Smoking-induced risk of leukemia. As cigarette smoke is a known source of benzene exposure [48], the reported increase in AML among smokers, with a relative risk ranging from 1.4 to 2.0, further supports a leukemogenic effect at low benzene exposure [182,183]. Cigarette smoke contains several carcinogenic agents in addition to benzene, and benzene's contribution to the increased risk has not been established. However, benzene in cigarettes contributes to an estimated 8–48% of smoking-induced leukemia deaths and 12–58% of smoking-induced deaths from AML [184]. Although far from being conclusive, several factors argue for benzene being an important contributor to smokers' leukemia risk, such as increased concentration of benzene, hydroquinone and catechol among smokers versus nonsmokers and similarities in the chromosome abnormalities found in smoking- and benzene-induced AML [183].

6. INFLUENCE OF POLYMORPHISM IN GENES ON THE SUSCEPTIBILITY TO BENZENE-INDUCED HEMATOTOXICITY

The concentration of benzene and its metabolites in biological media after a given level of exposure and the sensitivity to the toxic effects of benzene differ between individuals. The variability in the toxicokinetics is caused by biological factors such as race, sex, age and amount of adipose tissue and environmental influences such as routes of exposure, physical activity, competitive metabolic interaction, smoking, alcohol consumption and dietary habits [185]. Individual differences in the sensitivity to the toxic effects are explained partly by polymorphisms of genes important in benzene metabolism, DNA repair or regulation of hematopoiesis.

6.1. Enzymes Involved in Metabolizing Benzene

Genetic variation resulting in increased activity of the activation enzymes cytochrome P450 2E1, microsomal epoxide hydrolase and myeloperoxidase and/or decreased activity of detoxification enzymes glutathione-*S*-transferase and NAD (P)H:quinone oxyreductase have all individually been associated with increased susceptibility to benzene's toxic effects. Genetic variation has been associated with leukemia [186,187], benzene poisoning [188,189], leukocyte toxicity [11,190], chromosomal aberrations [191] and affected metabolism [192,193].

Myeloperoxidase's ability to metabolize phenol and hydroquinone to toxic quinones is assumed to play a key role in benzene's hematotoxic effect. Mutant genotypes of myeloperoxidase have been reported to be associated with a reduced risk of acute leukemia development, explained by less myeloperoxidase activity and diminished ability to catalyze benzene [187], while the normal expressed genotype has been associated with a rise in chromosomal aberrations [191] and a greater decline in leukocyte count [11] among benzene-exposed workers. Myeloperoxidase polymorphism has also been associated with reduced risk of other types of cancer [194,195].

6.2. Cytokines

Studies suggest that soluble mediators are important in developing benzene-associated hematotoxicity. Lan *et al.* [196] investigated the frequency of single nucleotide polymorphisms (SNPs) for 20 candidate genes involving these (hematopoiesis regulatory genes: cytokines, chemokines and adhesion molecules) pathways in 250 benzene-exposed (mean 5.44 ppm) and 140 unexposed workers. Lan *et al.* described reduced peripheral blood counts of total leukocytes, granulocytes, lymphocytes, $CD4^+$ T cells, CD4/CD8 ratio, B cells, monocytes and platelets. A significant correlation between leukopenia and SNPs of the interleukins IL-1A, IL-4, IL-10, IL-12A and vascular cell adhesion molecule 1 genes was found. The authors reported that selected variants seemed to influence only granulocytes, whereas others altered cell types of both the myeloid and lymphoid lineage, suggesting effects that could extend to earlier progenitor and possibly stem cells. Another report described an association between SNPs in the tumor necrosis factor α promoter region and the development of myelodysplastic syndrome (benzene-induced dysplasia) in individuals exposed to high concentrations of benzene [197]. Taken together, these two reports suggest that cytokine-mediated growth regulation is involved in benzene-associated hematotoxicity. One possible mechanism could be that these soluble mediators mediate survival growth-enhancing signaling to transformed cells.

6.3. Genes Involved in the Repair Pathway

A third study of the same study population described above [196] found an association between benzene-induced hematotoxic effects and a number of SNPs in seven genes important in repairing DNA double-strand breaks [198]. Among exposed workers, one SNP in BRCA2, four SNPs in WRN and one SNP in TP53 were associated with a decrease in white blood cell counts. These three gene products play an important role in multiple mechanisms including DNA damage recognition, replication, recombination, repair and cell cycle regulation, all of which are critical to maintain genomic integrity. Further, genetic polymorphism in the gene X-ray cross-complementation group 1, which plays an important role in both base excision repair and single-strand repair [199], was associated with a higher frequency of chromosomal aberrations and micronuclei in a group of benzene-exposed refinery workers [191].

7. CONCLUDING REMARKS

Although the association between benzene exposure and AML has been established, which exposure level that causes an increased risk and which exposure pattern that best predicts the risk (cumulative versus peaks) are still uncertain. No clear evidence indicates a threshold level below which benzene does not affect human hematopoiesis or peripheral blood cell levels. Emerging knowledge of nonlinear dose-related production of major metabolites, favoring the production of the hematotoxic quinones at low benzene exposure, implies a probable underestimation in previous risk assessments of the risk at low exposure.

The scientific literature also debates the association between benzene exposure and other malignancies of the blood and blood-forming organs. Although increasing evidence indicates an association between benzene exposure and multiple myeloma, evidence for other types of chronic leukemia and lymphoma is weak. There is a biologically plausible basis for suggesting benzene as a causal factor for these malignancies, and this is especially true for malignancies of the blood and blood-forming organs developing in the bone marrow, such as multiple myeloma, acute lymphoblastic leukemia and chronic myeloproliferative disorders. The bone marrow may be particular prone to benzene-induced toxicity through the presence of myeloperoxidase, an enzyme involved in forming hydroquinone, a biologically reactive benzene metabolite. These benzene metabolites are assumed to exert their effect through a concerted action of genotoxic damage, DNA repair failures and altered oncogenic signaling. Studies of benzene-exposed workers suggest that the risk of developing hematotoxicity also depends on genetic polymorphisms in benzene-activating and detoxifying enzymes, DNA repair capacity and various growth-regulatory soluble mediators. However, additional studies of the leukemogenic effects of benzene are definitely needed, including investigations of the effects on various hematopoietic progenitor cell subsets.

Together these observations suggest that, even at low exposure levels, benzene may contribute to the risk of malignancies of the blood and blood-forming organs, especially among genetically susceptible individuals. More knowledge about how benzene exposure affects the blood cells of the human system, such as affected signaling pathways or changes in gene expression, might provide hematologists with a basis for developing detection and technologies for preventing benzene-induced hematotoxicity.

Although the fraction of malignancies of the blood and blood-forming organs that can be attributed to occupational benzene exposure is probably low in the general population, it involves high risk for various groups of workers who are unwillingly subjected to the additional burden of this exposure. Exposed workers might be subjected to an unacceptable risk that can be avoided by enforcing proper preventive measures.

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