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BENZENE

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

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5. SUMMARY OF DATA REPORTED

5.1 Exposure data

Benzene is the simplest aromatic hydrocarbon. It is a volatile and ubiquitous air pollutant, mainly arising from anthropogenic sources such as combustion processes. It is found in crude oil and hence in petroleum products. Historically, benzene was used as a solvent, for example in glues and paints, and also in the rubber and chemical industries. At the present time, benzene is a high production volume chemical, despite being banned in consumer products in many countries. Its primary use is in the synthesis of ethylbenzene, used in plastics manufacturing.

Benzene exposure may occur in several industries and occupations, including the petroleum, chemical, and manufacturing industries, and coke making. In low- and middle-income countries, exposure may still occur in several industrial sectors, including shoemaking, painting, printing, and rubber product manufacturing. It is a component of gasoline, vehicular exhaust, industrial emissions, and tobacco smoke, all sources of environmental exposure.

Exposure to benzene mainly occurs via inhalation, but skin absorption is also possible. Benzene can be measured in both workplace and community settings using air monitoring and personal monitoring as well as biomonitoring, capturing all routes of exposure. Urinary *S*-phenylmercapturic acid (SPMA) and unmetabolized benzene in blood and urine are specific biomarkers, whereas *trans*,*trans*-muconic acid is not. The current preferred method for biomonitoring is to measure urinary benzene and SPMA; these measurements are non-invasive and reflect daily benzene exposure.

Occupational exposure to benzene is regulated in many countries, and there are also some environmental guidelines for benzene in air and drinking-water.

Full-shift occupational exposures in high-income countries are usually less than 1 ppm (3.19 mg/m^3) for most industries and occupational groups, including the upstream and downstream petroleum industry and automobile repair, and for diverse workers exposed to vehicle exhausts. However, workers in most of these industries may conduct short-term tasks that possibly result in exposure to high concentrations of benzene, such as maintenance activities where pipelines are open, tank cleaning, or top filling of road tankers with gasoline. Data from low-and middle-income countries are sparse; however, exposures considerably higher than those described above have been reported from China.

Environmental air levels, as determined at fixed monitoring sites, are generally orders of magnitude lower than occupational exposures. There is evidence that outdoor air levels have declined significantly over time in both Europe and the USA, where annual average concentrations are currently less than 5 μg/m^3; however, higher concentrations are measured in some cities in other regions of the world.

For occupational cancer epidemiology, high-quality exposure assessments use benzene measurements to derive individual exposure estimates. In studies of occupational exposures, these estimates represent long-term exposure in the workplace. Participants should have complete and detailed job histories for which the measured data are applied. For environmental (air pollution) epidemiology, exposure assessment typically relies on measurements of benzene in outdoor air collected from routine monitoring stations, or from modelled ambient concentrations for geographically defined gridded areas. These spatially referenced data for temporally relevant critical windows are linked to geocoded residences of study participants and used to generate individual-level estimates of benzene exposure.

5.2 Human carcinogenicity data

5.2.1 Acute myeloid leukaemia

The classification of benzene as a Group 1 carcinogen in previous *IARC Monographs* was based on sufficient evidence of an association between benzene exposure and risk of acute myeloid leukaemia (AML) and/or acute non-lymphocytic leukaemia (ANLL). This conclusion was supported by several occupational cohort studies that collected quantitative exposure data, revealing exposure–response trends between benzene exposure and AML and/or ANLL. According to the recent WHO classification of AML, related neoplasms are included in this category as AML not otherwise specified (e.g. pure erythroid leukaemia, acute megakaryoblastic leukaemia, and acute monocytic leukaemia). The following discussion referring to AML therefore includes ANLL.

Occupational and general-population studies published since the previous *IARC Monographs* on benzene, including two studies in occupational cohorts with careful assessment of benzene exposure, confirm the association between AML and exposure to benzene, and also demonstrate an exposure–response trend with quantitative exposure metrics.

5.2.2 Chronic myeloid leukaemia

Several cohort studies in the petroleum industry and other settings demonstrated increased risks for chronic myeloid leukaemia (CML). Other studies showed no evidence of an association, including two studies that were previously included in *IARC Monographs* volume 100F with quantitative estimates of exposure to benzene but did not report any exposure–response relationship.

An elevated risk of CML was reported in two new publications of occupational cohort studies with extended follow-ups, and a significant exposure–response trend was seen in the study that evaluated exposure–response. Among the four studies judged to be the most informative by the Working Group, the point estimates were above the null for all; however, only three studies included 6 or more exposed cases. The Working Group further noted a lack of clear evidence of an exposure–response gradient in the four available studies. Other co-exposures were present, but the potential for confounding could not be assessed.

5.2.3 Non-Hodgkin lymphoma

The broad category of non-Hodgkin lymphoma (NHL) includes chronic lymphocytic leukaemia (CLL), multiple myeloma (MM), and acute lymphocytic leukaemia (ALL), as well as follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and hairy cell leukaemia. In considering the data available at the time, the Working Group of *IARC Monographs* Volume 100F concluded that there was *limited evidence* in humans that benzene causes NHL. The current Working Group

examined all of the pertinent studies published before and after Volume 100F. In doing so, the Working Group assessed the quality of the old and new studies and noted that several high-quality cohort studies provided data for NHL in occupational settings and in the general population. These studies showed elevated relative risks for NHL as categorized in the studies, which were statistically significant in two studies. Two of these studies were conducted in China, and with relatively high levels of exposure to benzene in one occupational cohort. The studies that reported on NHL used different classifications of lymphoma, which varied over time and between studies. The Working Group therefore noted that associations were observed between benzene exposure and a heterogeneous classification of NHL.

CLL is currently included as a subgroup of NHL, but in the past it was generally considered as a separate entity (not always reported as such in papers). As noted in *IARC Monographs* Volume 100F, CLL can be an indolent disease of the elderly; this raises questions about cohorts that are not followed up until the study population is relatively old, and about studies that use mortality instead of incidence data. The diagnostic accuracy of CLL has also improved over time. Because of these concerns, the Working Group accorded the greatest weight to recent studies and those that reported incidence data; these included four studies (two occupational cohorts, and case–control studies in Italy and China) that used current classifications for lymphomas, including CLL. Three of these studies (three occupational cohorts) found positive associations between CLL and exposure to benzene, but the 95% confidence intervals included the null. Confounding from other occupational exposures was judged to be unlikely in these studies.

MM was considered separately in one case–control study and in nine occupational cohort studies. The numbers of exposed cases were generally small. Elevated relative risks were observed in four studies. The remaining studies did not find robust positive associations, but some showed elevated risks in the exposure category of highest concentration.

In *IARC Monographs* Volume 100F, the evidence of an association between benzene and ALL in adults was regarded as limited, based on a few occupational cohorts that included very small numbers of exposed cases and reported increased risks that were not statistically significant. Data for adult ALL and benzene exposure remain sparse: only one occupational cohort study has reported on ALL after the publication of the previous review. That study reported a non-significantly elevated risk based on a few incident cases, and did not provide exposure–response results. Among all of the included studies of adult ALL, the magnitude of the risk ratio estimates ranged from 0.8 to 4.5, and all confidence intervals included the null.

Other specific subtypes of NHL were reported in a few studies, including outcomes such as DLBCL, follicular lymphoma, and mantle B-cell lymphoma, but results were inconsistent.

Overall, the Working Group concluded that the evidence of carcinogenicity for NHL is *limited*; however, a small minority of the Working Group concluded that the evidence of carcinogenicity is *sufficient* for NHL.[1]

[1] The meta-analysis of five incidence studies revealed a statistically significant association between benzene exposure and CLL (pooled relative risk estimate, 1.53; 95% CI, 1.04–2.25). The relative risk estimates of four of these five studies were above 1.6; only one study found a slightly lower risk estimate of 1.40 (95% CI, 0.90–2.19). Therefore, the CLL risk is comparable to the AML risk (pooled risk estimate, 1.45; 95% CI, 0.96–2.17 when combining the six incidence studies). Chance and confounding can be ruled out with reasonable confidence. *(continued on next page)*

5.2.4 Childhood cancer

Information about a possible association between environmental benzene exposure and childhood cancer derives mainly from case–control studies. Seven studies investigated leukaemia, two studies reported on tumours of the central nervous system, and there were single studies published for each of lymphoma, Wilms tumour, retinoblastoma, and neuroblastoma. The seven studies reporting on leukaemia were inconsistent in the definition of leukaemia (including a variation over all leukaemias, acute leukaemia, ALL, and AML) and reported heterogeneous results for association with benzene exposure. The Working Group noted that all four studies reporting separate results for ALL and AML showed associations between benzene and AML in children (although most associations were not statistically significant), but no or weaker associations with ALL. Parental exposures to benzene before or during pregnancy were also considered in several studies of both cohort and case–control format. The Working Group noted a consistent association between exposure to benzene and AML for children, and coherence with findings for adult AML and benzene exposure, but could not rule out chance, bias, and confounding as alternative explanations.

5.2.5 Cancer of the lung

Several epidemiological studies of workers exposed to benzene have examined cancer of the lung. The most informative studies, which include those with larger cohort sizes, longer follow-up times, and either larger numbers of workers exposed to high concentrations or better-quality exposure assessments, have all reported statistically significant excesses of cancer of the lung among workers exposed to benzene. Positive trends between cumulative exposure to benzene and cancer of the lung were reported in two of these studies. However, none of these studies controlled for potential confounding by smoking or by occupational exposure to other lung carcinogens. The Working Group noted that smoking is a strong risk factor for cancer of the lung, and an important potential confounder of this association; in addition, the workers in these cohorts were potentially exposed to other occupational lung carcinogens.

Overall, the Working Group concluded that the evidence of carcinogenicity for the lung is *limited*; however, a small minority of the Working Group concluded that the evidence of carcinogenicity is *inadequate* for the lung.

5.2.6 Other cancers

Occupational cohort studies also reported data for several other cancer types and tumour sites, including cancer of the: nasal cavity, pharynx, larynx, and related sites; oesophagus;

(continued from previous page) Most studies show a positive association between benzene exposure and NHL incidence. Among the incidence studies, there are three high-quality cohort studies, which reveal clearly increased NHL risks: one found a relative risk of 3.90 (95% CI, 1.31–11.57) for ever versus never exposure to benzene (not including CLL and MM), another reported a hazard ratio of 1.86 (95% CI, 1.17–2.96) including MM but not CLL, and a third reported a hazard ratio of 1.49 (95% CI, 0.90–2.47) for the whole NHL group (including MM and CLL).
Most older studies, as well as some newer studies, did not include MM and CLL in the NHL group. However, the inclusion of CLL and MM in the NHL entity in these studies should not have changed the finding of a clear association between benzene and NHL, for the following two reasons. First, studies allowing for a direct comparison between MM and the (remaining or total) NHL group give no overall indication for lower MM risks compared with NHL risks as a whole. Second, as noted above, CLL risks are also clearly elevated.

stomach; colon, rectum, and anus; pancreas; kidney; liver and biliary tract; prostate; bladder, brain, and central nervous system; and skin. Each of these cancers was addressed in a small number of studies. For each cancer site, results were inconsistent across studies, exposure–response data were generally lacking, and potential confounding from other occupational exposures and behavioural factors was typically not controlled.

5.2.7 Quantitative data

Meta-regression analysis of data from six occupational cohort studies strongly supported a linear exposure–response relationship for AML and cumulative exposure to benzene.

5.3 Animal carcinogenicity data

There were 17 studies that reported on the effects of benzene inhalation in male and female mice. Several studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in mice exposed to benzene.

In one study in male and female mice, benzene caused significant increases in the incidence of myelogenous neoplasms (myeloid leukaemia) and solid tumours (other than of the liver or lymphomas) in males and females. In a second study in male mice, there was a significant increase in the incidence of solid tumours (other than of the liver or lymphomas). In a third study in male mice, there was a significant increase in the incidence of malignant lymphoma, squamous cell carcinoma of the preputial gland, carcinoma of the Zymbal gland, squamous cell carcinoma of the forestomach, and adenoma of the lung. In a fourth study in male mice, there was a significant increase in the incidence of lymphoma of the thymus gland (with a significant positive trend), and neoplasms of the haematopoietic and lymphoid tissues. In a fifth study in male mice, there was a significant positive trend in the incidence of lymphoma of the thymus gland. In a sixth study in male mice, there was a significant increase in the incidence of neoplasms of the haematopoietic tissues. In a seventh study in male mice, with a short duration of exposure, there was a significant increase in the incidence of adenoma of the lung. In an eighth study in male mice, there was a significant increase in the incidence of leukaemia or lymphoma (combined) and of adenoma of the lung. In a ninth study in male mice, there was a significant increase in the incidence of carcinoma of the Zymbal gland. Seven other studies were negative. One study was considered inadequate for the evaluation.

There were four oral administration (gavage) and two intraperitoneal studies of benzene in male and female mice. Some studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in mice exposed to benzene.

In a first study in which benzene was administered by gavage, benzene caused a significant increase in the incidence of the following lesions in males and females: bronchioloalveolar adenoma and carcinoma, hepatocellular adenoma or carcinoma (combined), squamous cell carcinoma of the Zymbal gland, adenoma or carcinoma (combined) of the Harderian gland, lymphoma or leukaemia (combined), and squamous cell papilloma of the forestomach. In males only, benzene caused a significant increase in the incidence of hepatocellular carcinoma, adenoma of the Harderian gland, carcinoma of the preputial gland, squamous cell papilloma or carcinoma (combined) of the forestomach, and pheochromocytoma of the adrenal gland. In females only, benzene induced hepatocellular adenoma, carcinoma of the Harderian gland, tubular adenoma of the ovary, mixed tumours (benign) of the ovary, tumour (benign or malignant) of the granulosa cell, and carcinoma and carcinosarcoma of the mammary gland.

In a second gavage study, benzene caused a significant increase in the incidence of tumours of the lung in males and females, and of carcinoma of the mammary gland in females. In a third gavage study, there was a significant increase in the incidence of tumours of the lung and leukaemia in males and females, and of carcinoma of the mammary gland in females. In a fourth gavage study in male and female mice, strain A/J, benzene induced a significant increase in the multiplicity of adenoma of the lung in males.

In a first study in which benzene was administered by intraperitoneal injection, there was a significant increase in the incidence and multiplicity of adenoma of the lung in male A/J mice but not in females. In a second (transplacental) study there was a significant increase in the incidence of tumours of the liver in the male offspring, and of lesions of the haematopoietic and lymphoid tissues (hyperplasia, myeloproliferative disorders, and myeloid/lymphoid neoplasia, combined) in the female offspring of pregnant mice given benzene intraperitoneally.

There were five studies of the carcinogenicity of benzene in rats: four oral administration studies (by gavage of males and females of different strains, i.e. Sprague-Dawley, Wistar, and F344) and one inhalation study in Sprague-Dawley rats (in pregnant females and their male and female offspring). All studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in rats exposed to benzene.

Benzene significantly increased the incidence of carcinoma of the Zymbal gland in male and/or female rats in four gavage studies, and in male and female offspring in a study of transplacental exposure followed by inhalation. It also significantly increased the incidence of squamous cell carcinoma of the oral cavity (including lip and tongue) in males and females in two gavage studies, and in the female offspring in the study of transplacental exposure followed by inhalation. Exposure to benzene significantly increased the incidence of carcinoma in situ of the forestomach in females and of acanthoma or squamous cell dysplasia (combined) of the forestomach in males and females in one gavage study, and carcinoma in situ of the forestomach in the female offspring in the study of transplacental exposure followed by inhalation. A significantly increased incidence of hepatocellular carcinoma was observed in the female offspring in the study of transplacental exposure followed by inhalation. Benzene caused a significant positive trend in the incidence of tumours of the haematopoietic and lymphoid tissues in males in one of the gavage studies, and a significant increased incidence of those same tumours in female offspring in the study of transplacental exposure followed by inhalation. There were also significant increases in the incidence of carcinoma of the skin in males in two gavage studies and of stromal polyps of the endometrium in females in one gavage study.

There were 12 studies that reported on neoplasms and preneoplastic effects induced by benzene (three whole-body inhalation, three oral administration (gavage), and six skin application studies) in one or both sexes of four different genetically modified mouse models of different genetic backgrounds. It was demonstrated that benzene induced cancer in different tissues (including tumours of the haematopoietic and lymphoid tissues) of genetically modified mice, depending upon the route of exposure.

In inhalation studies, B6.CBA-*Trp53*^{tm1Sia} haploinsufficient congenic inbred mice showed significant exposure-related increases in the incidence of lymphoma of the thymus gland in one study; C3.CBA-*Trp53*^{tm1Sia} congenic mice demonstrated a significant exposure-related increase in the incidence of lymphoma of the thymus gland, non-thymic lymphoma, and myeloid leukaemia in another study. One inhalation study in C57BL/6 h-Trx-Tg mice was negative.

In studies of B6.129-*Trp53*^{tm1Bra} N5 haploinsufficient mice exposed to benzene by gavage, increases in the incidence of sarcomas of the subcutis were observed in one study and atypical hyperplasia of the thymus gland in another. In another model of a haploinsufficient mouse with tumour-suppressor gene (the B6.129-*Cdkn2a*^{tm1Dep} congenic), oral exposure to benzene by gavage was associated with a significant dose-related increase in malignant lymphoma in males, but not in females.

Benzene application to the skin of female v-Ha-*Ras* mice resulted in a significant and rapid development of exposure-related squamous cell papillomas of the skin in one study, and of a significant increase in the incidence of granulocytic leukaemia in another; all other skin application studies were negative or inadequate for the evaluation.

5.4 Mechanistic and other relevant data

Benzene is well absorbed via inhalation as well as by oral and dermal exposure in all species studied, including humans and rodents. Benzene is widely distributed in the body by blood circulation; unchanged benzene is largely excreted by exhaled breath, with small amounts appearing in urine. The initial step of metabolism is oxidation to benzene oxide by cytochrome P450. Subsequent metabolism is complex, and includes the creation of a multiplicity of reactive electrophiles via multiple metabolic pathways in multiple tissues, including bone marrow. Major urinary metabolites detected in exposed humans include phenol, hydroquinone, catechol, (*E,E*)-muconic acid, and SPMA. There are some data suggesting increased metabolism at exposure to low concentrations, but these data are not definitive. Electrophiles are generated during benzene metabolism, as indicated by metabolite profiles and the production of epoxide- and benzoquinone-protein adducts in individuals exposed to benzene. There is *strong* evidence, including in exposed humans, that benzene is metabolically activated to electrophilic metabolites. There is *strong* evidence, including in exposed humans, that benzene induces oxidative stress and associated oxidative DNA damage. Several studies in exposed humans reported that exposure to benzene is associated with markers of oxidative stress, such as decreased serum glutathione levels, increased lipid peroxidation, increased reactive oxygen species, oxidative protein damage, and/or decreased antioxidant capacity. In addition, multiple studies in exposed humans reported oxidative DNA damage in the form of 8-hydroxy-2′-deoxyguanosine. Benzene or its metabolites induced oxidative stress in human and other mammalian cells in vitro, and in various tissues, including bone marrow, in mice.

There is *strong* evidence, including in exposed humans, that benzene is genotoxic, inducing DNA damage and chromosomal changes. Benzene induces DNA strand breaks and gene mutations in occupationally exposed humans, and DNA damage in human cells in vitro. In experimental animals exposed in vivo, benzene induced DNA adducts in bone marrow and leukocytes. Benzene metabolites induced benzene-derived DNA adducts in several studies in human haematopoietic cells. The multitude of studies of chromosomal end-points in humans exposed to benzene is largely consistent with respect to the induction of chromosomal aberrations and micronuclei. Specific cytogenetic changes have also been observed in exposed humans, including aneuploidy, translocations, and various other structural chromosome changes. Furthermore, in human cells in vitro, benzene with metabolic activation and benzene metabolites consistently induce chromosomal alterations.

The evidence is *strong* that benzene alters DNA repair or causes genomic instability, inhibiting topoisomerase II, which is involved in DNA

replication. No data on topoisomerase II were available in exposed humans. Benzene metabolites, particularly 1,4-benzoquinone and hydroquinone, directly inhibited topoisomerase II in human cell systems and in exposed mice.

The evidence is *strong* that benzene is immunosuppressive, including in exposed humans. Although no studies in humans were available that directly examined changes in immune function, many studies in exposed humans have demonstrated haematotoxicity, from decreased leukocyte counts at lower exposures to aplastic anaemia and pancytopenia at higher exposures. Specifically, reduced numbers and/or maturity of B-lymphocytes and CD4+ T-lymphocytes have been reported in multiple studies in exposed humans. Multiple experimental animal studies have demonstrated consistent immunosuppressive effects on assays for humoral and cell-mediated immune function, in addition to haematotoxicity, consistent with studies in exposed humans. In addition, several studies have found that haematotoxicity induced by benzene, at various levels of severity, has been associated with a future risk of developing a haematological malignancy or related disorder.

Haematotoxicity observed in exposed humans and experimental animals provides indirect evidence that benzene exposure leads to alterations of cell proliferation and cell death. In human cells in vitro, benzene or its metabolites induced apoptosis consistently across multiple haematopoietic cell types, which could be prevented by induction of the detoxifying enzyme NAD(P)H quinone oxidoreductase 1. In addition, in mice, benzene depressed the cycling fraction of bone marrow cells/progenitor cells mediated by Trp53, and induced apoptosis in various mouse haematopoietic cells in vivo and in vitro. After cessation of benzene exposure, dynamic recovery proliferation of bone marrow cells/progenitor cells was observed. Overall, the evidence is *strong* that benzene alters cell proliferation, cell death, or nutrient supply, specifically with respect to induction of apoptosis.

The evidence is *strong* that benzene modulates receptor-mediated effects, specifically with respect to aryl hydrocarbon receptor (AhR). No data on AhR were available in exposed humans or in human cells. Benzene does not induce haematotoxicity in AhR-knockout ($AhR^{-/-}$) mice, or in wildtype mice whose marrow cells were repopulated with cells from $AhR^{-/-}$ mice after irradiation. Benzene and its metabolites hydroquinone and *p*-benzoquinone did not directly activate AhR in vitro in mouse hepatoma cells.

There are few data on the remainder of the 10 key characteristics of carcinogens (induces chronic inflammation, induces epigenetic alterations, or causes immortalization).

In the ToxCast/Tox21 high-throughput testing programmes of the United States government, four metabolites of benzene (phenol, catechol, hydroquinone, and 1,4-benzoquinone) were individually tested in several assays in vitro that have been mapped to the key characteristics of carcinogens. Few of these assays demonstrated metabolic capacity. Phenol was largely inactive, while the activity of the other three metabolites for oxidative stress and AhR corroborated other mechanistic data on these key characteristics. 1,4-Benzoquinone was also active in many assays mapped to inflammation.

Studies in exposed humans examining exposure–response gradients were available for the end-points of micronucleus formation, chromosomal aberrations, and leukocyte counts. In the majority of studies examined, an exposure–response gradient was reported.